

THE EFFECTS OF INDUCED HYPERTHERMIA

IN ADVANCED MALIGNANT DISEASE

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M.D. THESIS

UNIVERSITY OF EDINBURGH

1977



DECLARATION

This thesis was written by myself based on work carried out over the past five years. I was a member of the team involved in the treatment of advanced cancer by whole body hyperthermia. I was responsible for investigation of the physiological background necessary to enable a safe method of whole body hyperthermia to be established, and for evaluating the response of patients to this form of therapy.

The second part of this thesis is based on a new method of hyperthermia perfusion of the urinary bladder for the treatment of transitional cell carcinoma. I devised the apparatus and carried out all the treatments in this section. I assessed all the bladders before and after treatment and took most of the biopsies.

In any new treatment of patients, one person can only be part of a team concerned with the best possible treatment for each patient. I would like to thank all my colleagues who, by their help, have made this thesis possible.

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SECTION 1.

SUMMARY

- 1.1 Fever therapy in the treatment of cancer is reviewed. Biochemical mechanisms of heat induced cellular damage are discussed with ways of potentiating the selective lethal effect. Conditions inducing heat resistance are stated.
- 1.2 Relationship of temperature to time above the 'critical temperature' for cell death is described. A clinical unit of heat stress, the centigrade degree minute is defined and used in subsequent studies.
- 1.3 Animal work involving treatment by temperatures in excess of 40°C. (hyperthermia), is reviewed. Methods of inducing controlled whole body hyperthermia are described, and the difficulties discussed. Dr. Pettigrew's method is described.
- 1.4 Physiological studies during hyperthermic therapy of disseminated human cancer investigating the haemodynamic response, fluid and electrolyte losses, and modifications of replacements required to produce a stable, safe treatment are described.
- 1.5 Liver sensitivity limited the maximum therapeutic temperature. Increasing stress and a leucocytosis was observed during treatment. Prolonged treatment in sensitive tumours induced disseminated intravascular co-agulation necessitating fractionation of therapy.
- 1.6 Tumour response to treatment is assessed and complications noted. Whole body hyperthermia

alone was not curative but caused good short term palliation of sensitive tumours.

1.7 Methods of inducing local hyperthermia are evaluated.

1.8 A new method involving hyperthermic perfusion of the distended urinary bladder for transitional cell carcinoma is described. Advantages including tumour exposure, heat gradient and reproduceable effects are discussed.

1.9 Following perfusion under epidural anaesthesia the damage to tumour and normal bladder was proportional to the treatment given; that necessary for deep tumour necrosis produced unacceptable damage to the normal bladder. Therapy was limited by vascular thrombosis in the bladder wall.

1.10 Treatment given by smaller daily fractions did not summate. Treatment without epidural anaesthesia was ineffective and the reasons for this are discussed. Vascular damage was of use in arresting gross haematuria.

1.11 Hyperthermia alone was palliative but combination with radiotherapy or chemotherapy may be curative.

SECTION 2INTRODUCTIONA. INTRODUCTION.B. A HISTORICAL REVIEW OF FEVER THERAPY.C. BIOCHEMICAL MECHANISM OF ACTION OF
HYPERTHERMIA.

- INHIBITION OF OXYGEN UPTAKE
- INHIBITION OF NUCLEIC ACID SYNTHESIS
- CHANGES IN THE NUCLEOLUS AND RNA SYNTHESIS
- EFFECTS ON RIBOSOMAL FUNCTION AND PROTEIN SYNTHESIS
- EFFECTS OF CHROMOSOMES AND THE CELL CYCLE.
- EFFECTS ON CELL MEMBRANES
- EFFECTS ON LYSOSOMES
- INHIBITION OF REPAIR
- SUMMARY OF BIOCHEMICAL MECHANISMS OF ACTION

D. FACTORS AFFECTING HEAT SENSITIVITY.E. ACQUIRED RESISTANCE.F. RATE OF CELL DEATH RELATED TO TEMPERATURE
AND TIME.G. ANIMAL STUDIES IN VIVO.

A. INTRODUCTION

- 2.1. Surgical excision of localised cancer is curative. Cancer therapy for extensive or inoperable disease relies on the narrow margin of difference shown in respect of recovery from stress of the normal and the cancer cell. Such stress may be applied by selective poisons, localised ischaemia, X-ray therapy or a combination of these agents. Recently there has been considerable interest in the response of cancer cells to heat stress.
- 2.2. In this thesis the background of cancer therapy by heat stress using temperatures in excess of 40°C. (hyperthermia) is reviewed, together with in vitro and animal studies which illustrate the mechanism of action of hyperthermia and its selective effect on some cancer cells. Two clinical studies were carried out to assess the effect of hyperthermia in the treatment of human cancer. The first using whole body hyperthermia for disseminated disease and the second, hyperthermic perfusion of the urinary bladder in the treatment of localised transitional cell carcinoma. The results of these studies are tabulated and show that hyperthermia alone can offer a degree of palliation in some advanced cancers but is not curative. Future management using hyperthermia as a potentiating agent to conventional therapy, in selective tumour types, has a great promise. The effect of hyperthermia alone, its fractionation, dosage and the safety margins in man are important.

building blocks with which to plan this combined therapy.

B. A HISTORICAL REVIEW OF FEVER THERAPY

2.3 In 1866 Busch described the complete disappearance of a sarcoma from the face following recurrent attacks of erysipelas. The patient remained well and disease free when reviewed two years later. Bruns (1887) observed a terminally ill patient with a metastatic malignant melanoma who developed intercurrent erysipelas and associated pyrexia of 40°C. for several days. All the tumours disappeared and the patient was alive, well and free from disease eight years later.

2.4 The work of W. B. Coley in the New York Hospital has been described by his daughter (Nauts, Fowler and Bogatko, 1953). After losing one of his first patients with a sarcoma he reviewed all such cases treated in that hospital and found the only survivor had a severe post operative wound infection with erysipelas causing a prolonged high fever. In May 1891 Coley attempted to transfer erysipelas to a patient with a recurrent and inoperable myosarcoma of the neck. After repeated trials he succeeded and the resulting severe erysipelas was associated with complete regression of the tumour. In attempting to induce erysipelas

in 9 other patients Coley recognised the difficulties that either the patient might prove immune to infection or the infection might prove so severe as to be fatal. He reported this group of 10 patients in 1893 together with 28 who had accidentally developed intercurrent infection with erysipelas. All patients had histologically diagnosed advanced cancer. In 12 the tumours completely regressed and 19 patients showed some improvement. Of the 10 patients that Coley had deliberately infected the two patients with the highest fevers survived for 7 and 27 years respectively. Realising the dangers of live vaccines Coley then tried injecting streptococci sterilised by heating to 100°C. or by filtration. These proved entirely ineffective.

- 2.5 In December 1892 Coley combined toxins from bacillus prodigiosus with those of the streptococci in an attempt to produce a more effective product. The first Coley mixed toxins were sterilised by filtration and the first case treated by this preparation was a bed ridden young man with a sarcoma of the abdominal wall extending to the pelvis and involving the bladder. He was given repeated injection of toxin into the tumour over a 4 month period. Each injection produced a pyrexia with a rise of temperature between 0.5 and 0.6°F. His extensive growth

regressed completely and he eventually died free from disease, 26 years later (Nauts, Fowler and Bogatko, 1953).

2.6 From that time until his death in 1936 Coley tried a variety of toxins and in 1946 when Nauts reviewed 600 case histories from different clinics in America at least 15 varieties of the toxin had been used. The methods of treatment varied with different dosages, injection sites and duration of treatment, but the greatest response rate was noted with patients showing a marked response to toxin therapy associated with a temperature rise above 39.4°C . In a study reviewing 86 patients with lymphosarcoma treated by Coley's toxins Nauts and Fowler (1969), found that no patient survived who had little or no febrile reaction to the toxin, 11% with a moderate reaction and 48% of those with a marked reaction (averaging 38°C . to 40°C .) survived. The highest cure rate being in patients treated over a period of 4 - 6 months.

2.7 Coley's toxins were rarely, if ever, standardised biologically and consequently the effects were inconsistent and unpredictable, successful treatment often leading to haemorrhage and necrosis. In 1940 Shear and Perrault attempted to isolate the pyogenic and tumour necrotising substance and devise a method of bio-assay to

standardise its potency. The toxin was found to be a high molecular weight polysaccharide but its effect in transplantable sarcoma in mice was unpredictable. Further purified extracts of bacterial polysaccharides capable of producing pyrexia and tumour necrosis were obtained by Creech, Hamilton et al, in 1948 but the high toxicity of such extracts discouraged clinical studies (Rieman and Nishimura, 1949). The use of toxins fell into disrepute, partly because of the effects were unpredictable and had a considerable mortality when given to seriously ill patients with disseminated cancer, and partly because of the development of X-rays and radium, with their use in the treatment of neoplasms.

2.8 In 1918, Rhodenburg reviewed 166 cases of well documented spontaneous regression of human tumours and found 72 cases had intercurrent high fevers, heat applications or severe infections. Of 26 spontaneously regressing sarcomas, 19 had fever, heat treatment or infection. Everson and Cole, (1956) reviewed 1,000 cases of spontaneous regression reported in the world literature and mentions fever or infection as a possible factor of regression in 130 cases which he considered adequately documented.

2.9 In the early part of this century there was general agreement that in some cases infection or toxins might have a beneficial effect in the

treatment of cancer. It was not known if the effect due to hyperpyrexia, to a direct action of bacterial products, or to stimulation of the immune response. Vidal in 1910 observed that pyrexia had been associated with a large number of treatments causing cancer regression. He believed that leucocytosis and other symptoms of fever produced a profound constitutional reaction of the whole organism to the tumour. Recently there has been renewed interest in stimulating cell mediated immunity against tumours by activating thymus dependant lymphocytes with B.C.G. or corynebacterim parvum (Klein 1973, MacGregor and Falk, 1975).

- 2.10 In this thesis the effects of hyperthermia on physiology and the function of the normal and cancer cell are compared. The immunological response is at present under study but it is most probably that the overall effects are contributed, as suggested by Crile in 1963, by a combination of both direct damage with decrease in tumour bulk, and a subsequent stimulation of the immune response.

C. BIOCHEMICAL MECHANISMS OF ACTION OF HYPERTHERMIA

- 2.11 Lambert 1912 using hanging drop plasma cultures, found that mouse sarcoma cells survived for three hours at 43°C. whereas connective tissue

cells derived from mouse aorta, survived for six hours. Vollmar (1941) found that explants of normal chick and rodent tissue survived treatment at 42°C . while ascites and sarcoma explants did not. More recently Bender and Schramm (1966), estimated the temperature of a 30 minute exposure which produced irreversible damage in 46 tumours of animal and human origin and compared these with a variety of normal tissues. There was a tendency for the tumour cells to be more sensitive than normal with the difference being of the order of $1 - 2^{\circ}\text{C}$. In some instances there was no difference between tumour and normal sensitivities. Since that time numerous works have confirmed these studies (Levine and Robbins, 1970).

INHIBITION OF OXYGEN UPTAKE

Variation of thermal tolerance of normal tissue

- 2.12 In 1915 Fuhrman and Field found that the rate of oxygen uptake of rat liver slices increased with temperature up to 45°C ., whereas rat heart reached a maximum uptake of 38°C . then declined to half that value to 42.5°C . In a further study by Burger and Fuhrman in 1964, the rate of oxygen consumption was measured at 38°C . after exposure of various rat organs to hyperthermia for varying time intervals. In the liver there was a reduction of 20% after one hour at 45°C . Cerebral cortical tissue showed the same reduction after one hour at

43°C. and for kidney cortical tissue the same effect was elicited after one hour at 44°C.

2.13 Conversely when the whole animals were heated to between 43 - 44°C. until they became anoxic, the oxygen uptake of tissue slices showed no impairment in the cerebral cortex, whereas liver oxygen uptake was reduced by 40%. This resistance of cerebral cortex was confirmed and studies by Ten Carte (1949) by electro-encephalographic studies which demonstrated that the disappearance of the normal pattern only occurred at between 44 and 45°C. Mossa (1927) found that chick neurones maintained in culture survived up to temperatures of 49°C. As judged by inhibition of oxygen uptake, normal tissues vary in their resistance to damage produced by elevated temperature, but are in general, quite tolerant - the rat brain being remarkably resistant to thermal damage (Burger and Fuhrman, 1964).

Variation of oxygen uptake of tumour during hyperthermia.

2.14 Some tumour cells show a greater reduction in aerobic and anaerobic glycolysis with a moderate increase in temperature than normal cells. This was first clearly demonstrated by Westermarck in 1927 who compared Flexner Jobling carcinoma and Jensen sarcoma cells with normal rat liver.

Cavaliere, Ciocato et al (1967) repeated these experiments using Novikhoff hepatoma and Ehrlich ascites cells as compared with normal rat liver cells and found that the oxygen uptake of the tumour cells was considerably less than 42°C . than at 38°C . There was little difference in respiration at these two temperatures in normal or regenerating liver cells. The inhibition of respiration of the Novikhoff hepatoma cells was irreversible after 90 minutes. Anaerobic glycolysis was not inhibited in either groups of tissue until temperatures of between $42 - 43^{\circ}\text{C}$. were used.

2.15 Muckle and Dickson (1971) confirmed these results in the rabbit working with a VX2 carcinoma at 42°C . Following incubation at this temperature the tumour showed a decrease in viability and growth potential as measured by its ability to produce tumours when subsequently inoculated into healthy rabbits. They found respiration and anaerobic glycolysis of normal rabbit liver, kidney and red blood cells were unaffected by incubation at 42°C . Dickson and Muckle (1972) found that whereas the oxygen uptake of the VX2 carcinoma was inhibited by two hours at 42°C . cells from the metastases of the VX2 carcinoma showed some decrease but no inhibition of oxygen uptake at 42°C .

2.16 Dickson and Shah (1972) showed that there was depression of respiration in rat breast adenocarcinoma at temperatures over 40°C . with irreversible

damage to the cells after four hours as indicated by a low oxygen uptake, loss of viability and failure of the cells to proliferate.

INHIBITION OF NUCLEIC ACID SYNTHESIS

Normal Cells

2.17 The rate of synthesis of nucleic acids is usually measured by incubating cells with radioactively labelled precursor such as thymidine or uridine. The cells are then fixed and covered with a layer of photographic emulsion for a period of one month. When developed such autoradiographs show grains of silver which can be counted over a standard area of nuclei and converted to relative rates by comparison with the grains per unit area of the controls.

2.18 In 1965 Siskin, Morsca et al, exposed human amnion cells to various elevated temperatures for five hours and then measured the uptake of Tritium labelled thymidine. He found that the greatest rate of incorporation was in the range of 37 to 39°C. At temperatures above 40°C. there was a rapid fall in the rate of DNA production and at temperatures between 41 and 43°C. there was an arrest of production with extensive irreparable cellular damage. Simard and Bernhard, (1967), working with hamster fibroblasts and using high resolution autoradiography was able to demonstrate

a profound disturbance of RNA synthesis with little incorporation of tritiated thymidine following a hyperthermia episode. The nucleolus, which is the main site of RNA synthesis, was particularly sensitive being selectively destroyed after 15 minutes at 42°C.

Tumour Cells

- 2.19 Mondovi, Agro et al, (1969) working with Novikhoff hepatoma cells found a 50% reduction of incorporation of ^3H thymidine into DNA after one hour at 43°C. though inhibition was evident at temperatures over 40°C. In comparison an increased incorporation of precursors was found in regenerating rat liver cells after incubation at 43°C.
- 2.20 Warocquier and Scherrer, (1969) studied Hela cells which were less sensitive to heat and showed a different pattern. During incubation at 42°C. the rate of ^{14}C uridine uptake fell uniformly reaching a plateau at six hours. This was confirmed by Love, Soriano and Walsh, (1970) who also found that the net RNA synthesis was impaired though not completely blocked by this degree of hyperthermia but at 46°C. there was complete inhibition of nucleic acid synthesis.
- 2.21 Mondovi, Agro et al (1969) related the heat sensitivity to the 'malignancy' of the tumour, Novikhoff cells being more metabolically active,

were more easily inhibited than minimal deviation hepatoma 5123 cells. A critical temperature for Novikhoff hepatoma cells was 41°C . by increasing the temperature above this no additional effect was achieved. Wüst, Norpoth, et al (1973) found that there was inhibition of uptake of labelled precursors of nucleic acid synthesis in all proliferating tissue incubated at 41°C . They found no difference in sensitivity between Jensen's sarcoma, G.W.39 tumour, embryo tissue and regenerating liver cells. The degree of inhibition was proportional to the extent and duration of the hyperthermia. There were no corresponding influences of hyperthermia on non-proliferating tissues, the sensitivity to temperature appeared to be limited to proliferating cell systems.

- 2.22 These findings would correspond to Poswillo, and Nunnerly's (1974) finding that hyperthermia is a teratogenic agent in marmosets and is of clinical interest as a factor linking prenatal influenza with central nervous system defects in children (Edwards 1972).

CHANGES IN THE NUCLEOLUS AND RNA SYNTHESIS

Changes in the structure and function

- 2.23 Ribosomal RNA is synthesised mainly in the nucleolus, by transcription of nucleolar DNA,

forming a large precursor molecule of about 45s, which is then cleared through a series of intermediate steps to active 28s and 18s ribosomal RNAs. These combine with protein and move into the cytoplasm constituting the ribosomal sub-units. The active granular ribonuclear protein of the nucleolus is concerned with protein synthesis.

2.24 Following incubation of BHK cells derived from Syrian hamsters, at 42°C. Simard and Bernhard, (1967), found a loss of granular ribonuclear protein, intranuclear chromatin and nucleolar structure. The nucleolus became round and sometimes had wide open meshes and the fibrillar reticulin completely disappeared. After recovery from heat stress the cells built up their lost nucleolar structure in an exaggerated manner, the nucleolus becoming larger than previously with granular ribonuclear protein in greater amount. These findings were confirmed by Dickson and Shah (1972) in the VX2 rabbit carcinoma.

2.25 Love, Soriano and Walsh, (1970) working with BHK 21 cells, three lines of Hela cells and two lines of human diploid cells, found that the nucleolar DNA and granular DNA disappeared following exposure of exponentially growing cells to temperatures of 45 to 46°C. for periods of 15 to 16 minutes. Diffusion of the nucleolar material was demonstrated by specific stains to be throughout the nucleus. All such cells developed

cytoplasmic inclusion consisting of aggregations of ribosome like granules and no cytoplasmic inclusions were observed in cells that did not have nucleoli. At temperatures of 41 - 44°C. the changes occurred but were less marked than at 46°C.

- 2.26 Simard and Bernhard, (1967) noticed a critical temperature at 42°C. in BHK cells, the nucleoli rather suddenly becoming heat sensitive after 15 minutes at 42°C., though a more pronounced effect was noticeable after one hour.

EFFECTS OF RIBOSOMAL FUNCTION AND
PROTEIN SYNTHESIS.

- 2.27 McCormick and Penman (1969) found that the incubation of Hela cells at 42°C. caused a rapid disaggregation of polysomes and a corresponding reduction in the rate of amino acid synthesis. This was maximal after 9 minutes at 42°C. after which time there was a slow recovery of protein synthesising capacity in Hela cells. The protein produced at elevated temperatures was not normal and seemed to be produced by a thermally stable group of small ribosomes. Further more this recovery of synthetic power was dependant on the production of a species of RNA which promotes the association of ribosomes with messenger RNA. This species of RNA is produced by cells under

prolonged heat stress.

2.28 The process of ribosomal disaggregation caused by heat stress was reversible by subsequent incubation at 37°C., recovery being complete within 24 hours. Reversibility of the disaggregation of polysomes was confirmed by Love et al (1970) in Hela cells at 46°C. and Heine (1971) who observed complete recovery by 24 hours.

2.29 Mondovi, Agro et al (1969) found that in Novikhoff hepatoma 5123 cells there was a progressive inhibition and final arrest of protein synthesis at 40°C. whereas the rate of protein synthesis in regenerating liver cells was higher at 43°C. than at 38°C.

EFFECTS ON CHROMOSOMES AND CELL CYCLE

In Vitro Observations

2.30 Rao and Engelberg, (1965) working with Hela cells showed that exponential growth could be maintained between 33 - 40°C. The growth rate was maximal at 38°C. and fell rapidly above 40°C. There was no cell division at 41°C. but an increase in the mitotic index was observed. Sisken, Morasca and Kibby, (1965) found that the optimum temperature for human amnion cells was between 37 - 39°C. Some human tumour lines showed an arrest of growth occurring between 39

and 40°C. with a delay in mitosis with cells being arrested in metaphase. Above 40°C. irreparable damage to some tumour lines occurred while other lines showed thermal stability (Selawry, Goldstein and McCormick, 1957).

2.31 Westra and Dewey, (1971) found that heat stress has a differential effect on the various parts of the mitotic cycle. Synchronous populations of Chinese hamster ovary cells were heated in different phases of their cycle for 6, 10 or 15 minutes at 45.5°C. There was a dramatic decrease in survival as cells moved into the 'S' phase followed by an increased survival in G2 with an increased sensitivity in metaphase. Cells heated in mitosis induced tetraploidy in 90% of the cells scored in the next mitosis and 5% diplochromosomes were observed. There was a long mitotic delay of up to 11 hours induced by heat regardless of the phase heated, and it was possible to induce mitotic synchrony by phased heating, (Martin, 1964).

2.32 The 'S' phase was very sensitive for cell killing and cells heated in this phase sustained a very high frequency of chromosomal aberrations, which increased with the time of treatment (Dewey and Westra, 1971). Abnormalities produced included deletions of chromosomal material, chromatid deletions and chromatid interchanges. Half of the aberrations in the mitotic and G1

cells occurred at the position of the secondary constriction of the X chromosome. The high frequency of aberrations agreed qualitatively with the high sensitivity of cell killing in the 'S' phase.

- 2.33 The effect of inhibiting the synthesis of DNA or protein by cyclohexamide in Hela cells protected the cells from heat damage (Palzer and Heidelberger, 1973). Further protection was afforded when both DNA and protein synthesis were suppressed. Contrasting effects were obtained when RNA synthesis was blocked which produced an increase in cell killing during hyperthermia. Giovenella, Lohman and Heidelberger, (1970) found this same effect to be true in L.1210 cells.

EFFECTS ON CELL MEMBRANES

In Vitro Observations

- 2.34 Belehraddek (1957) suggested that the cause of 'heat death' in cells was due to melting of cellular lipids. This lipoid liberation theory was originally proposed by Heilbrunn in 1924 and was based on the relationship between the degree of saturation of the lipids in the tissues of a poikilotherm and environmental temperature. Low

environmental temperatures leads to the formation of unsaturated fatty acids with a low melting point, whereas at higher environmental temperatures a greater proportion of saturated fatty acids of higher melting point are laid down. The 'melting' point of the lipids varies with the degree of saturation but is critical to maintain the specific liquid crystalline state of cellular membranes. Experiments with goldfish have confirmed that a relationship exists between the acclimatised state of the fish and the degree of saturation of brain phospholipids (Johnston and Roots, 1964).

- 2.35 The permeability of cell membranes change during hyperthermia with an increase in permeability of the cytoplasmic membrane and the consequent passive leakage of solutes. This has been shown to be an exponential relationship with temperature as measured by efflux of flour-excein from dye loaded Erlich and Yoshida ascites cells (Strom, Santoro et al, 1973), ³H labelled uridine from pig kidney cells (Reeves 1971), loss of intracellular ions in the crayfish (Bowler, 1973), or reflux of Lanthanum across the damaged membrane (Fahimi and Contan, 1971).

EFFECTS ON LYSOSOMES

- 2.36 Overgaard and Overgaard (1972) working with

mouse mammary carcinoma cells, found an increase in the number and activity of lysosomes following incubation at 41 - 43.5°C. The cellular changes were similar to those following X-ray therapy and consisted of pronounced autophagy with destruction of cytoplasmic elements such as mitochondria, the golgi apparatus and in extreme cases, the nucleus. Turano, Ferraro et al, (1970) found that Novikhoff hepatoma and Erlich ascites cells have very labile lysosomes, being markedly more fragile at elevated temperatures than were lysosomes from normal regenerating liver cells. Overgaard and Overgaard, (1975) proposed that autodigestion was one method of action of hyperthermia. Heat alone increased lysosomal activity and by inhibiting aerobic glycolysis which together with increased anaerobic glycolysis caused an increase in lactic acid production by the cell. This resulted in a decrease in the intracellular P.H. which also stimulates lysosomal activity (Eigner, 1961).

- 2.37 As leakage of lysosomal enzymes due to membrane damage might cause an inhibition of oxygen uptake in the affected mitochondria, Turano et al (1970), incubated cells with trypan blue, which inhibits lysosomal enzymes, and showed no change in the inhibition of oxygen uptake at elevated temperatures. He therefore concluded

that the lysosome was not the primary site of action of hyperthermia though there were effects on the lysosomal membrane. Heat activation of lysosomes may be important in cell killing though autophagocytosis is a common response to many sublethal agents, and is an effective means of disposal of damaged cytoplasmic organelles without the death of the cell (Ericsson 1969).

INHIBITION OF REPAIR

2.38 Mammalian cells have the capacity to repair sublethal damage from noxious stimulæ (Elkind and Sutton, 1960). This property has been extensively studied following X irradiation, following which there is a characteristic shoulder in the survival of cells to a gradually increasing dose of X-rays prior to a region of exponential response in which dose and cell death rates are directly proportional (Puck and Marcus, 1955). The shoulder is due to the capacity of the cells to accumulate sublethal damage before a lethal effect is registered (Elkind and Sutton, 1960).

2.39 Repair enzymes, such as DNA polymerase, depend on protein stability for maintenance of a functional quaternary structure. In vitro work on thermal stability of complex enzymes

necessary for cellular repair have shown that they are only functional between narrow temperature limits and are readily denatured by temperatures of 42°C . or above (Horowitz and Leopold, 1951). The more complex molecules being more thermolabile (Brandts, 1967). DNA polymerase has been found to be reversibly temperature sensitive; it is inactive above 42°C . but becomes functional again when the temperature is lowered (Karam and Speyer, 1970). The heat inactivation energy causing denaturation of several cellular enzymes and proteins has been found to be between 110 and 198 K Cals/mole (Johnson, Eyring and Polissar, 1954), compared with an inactivation energy for the cell between 125 - 140 K Cals/mole (Westra and Dewey, 1971). This suggests that the denaturation of critical protein plays an important part in the heat inactivation of mammalian cells.

2.40 That the proteins inactivated are those normally involved in repair of sublethal damages is suggested by the loss of the shoulder on the survival curve when cultures are treated by hyperthermia combined with radiotherapy (Harisiadis, Hall et al, 1975), Ben-Hur, Elkind and Bronk, (1975) found complete inhibition of repair or radiotherapeutically induced lesions

by incubation at 42°C. for 1.5 hours. This inhibition of repair of cellular damage induced by X-rays has been shown in many tissues (Gillette and Thrall, 1974, Robinson, Wizenberg and McCreadie, 1974, Gerner, Connor et al, 1975), each tissue showing a differing thermosensitivity. The same pattern has been shown after exposure to chemotherapeutic agents (Palzer and Heidelberger, 1973, Hahn, 1974).

Factors Affecting Repair

- 2.41 Repair has been shown to be dependant on nutritional status, those cells cultured and starved during hyperthermia being less able to repair sublethal damage. This would account for the increased sensitivity of hypoxic tumour cells to heat (Kim, Kim and Hahn, 1976). Chinese hamster ovary cells treated by incubation at 45.5°C. for 7 - 9 minutes prior to irradiation showed complete inhibition of repair. The half value time for recovery from heat damage as measured by the return of the shoulder of the X-ray survival curve was 9 hours for cells in G₁, or 16 hours in cells in 'S' phase. This recovery of repair capacity occurred in the absence of cell cycle progression which was delayed by approximately 17 hours (Gerwick and Dewey, 1974).

SUMMARY OF THE BIOCHEMICAL MECHANISM
OF ACTION.

2.42 Hyperthermic stress is a general effect on the cell and there are changes in the function of most cellular activities. By an increase in temperature there is an increase in entropy and, hence, an increase in the rate of chemical reactions up to a critical value when the spatial arrangement of the complex substances taking part are changed. Each system has a different critical temperature whereas the survival of the whole cell is dependant not only on the damage incurred but its capacity to repair sublethal damage. The longer the cell is stressed the more damage is done, this taken together with some inhibition of the repair mechanism, results in a temperature time survival curve characteristic for that cell in a particular environment. Figure 1. summarises the reported effects of hyperthermia stress and emphasises the fact that cellular enzyme systems are intimately linked with reticulo endothelium and the interdependence of both enzyme and membrane.

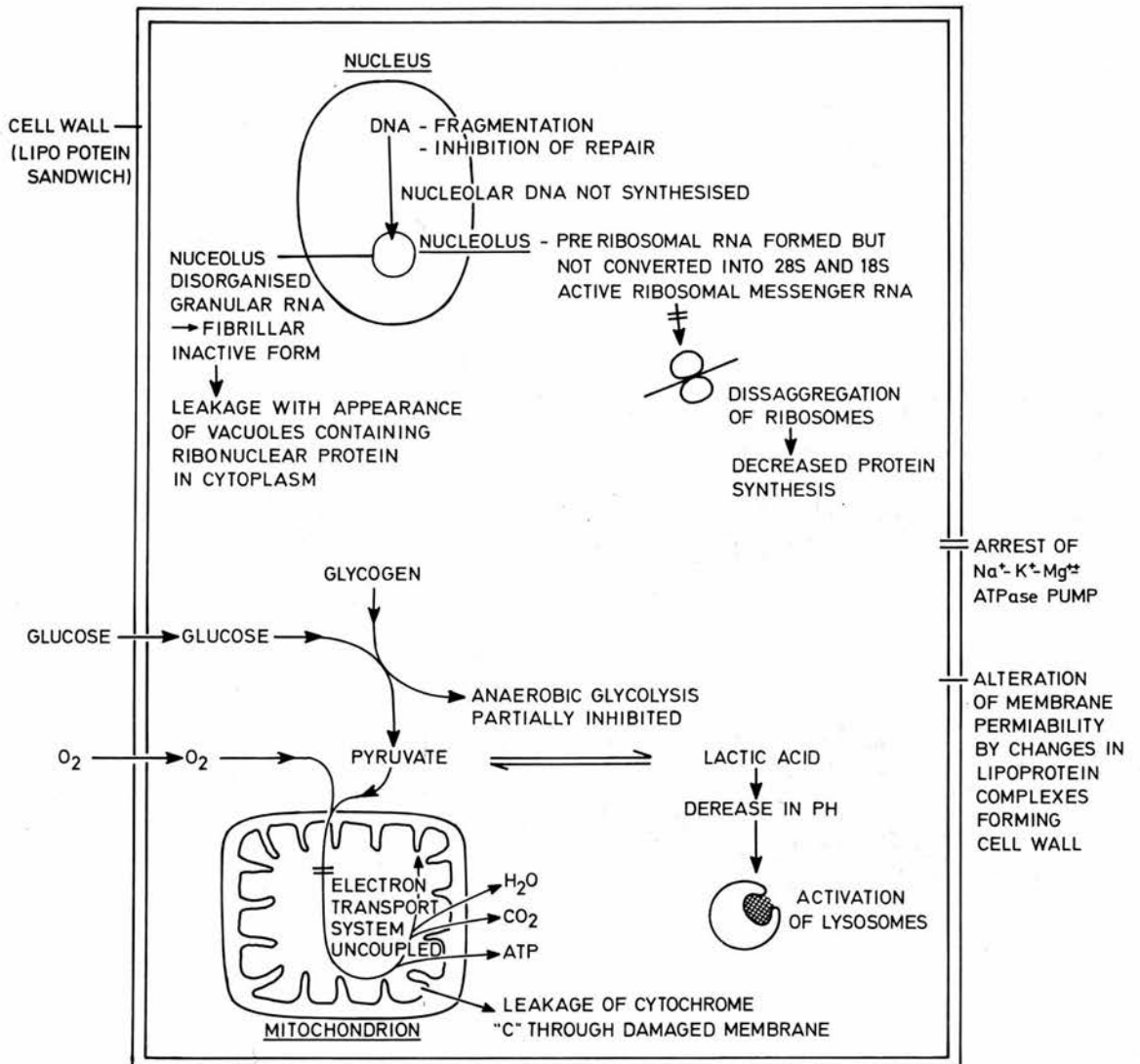


FIGURE I

Summary diagram of the biochemical mechanisms of action of hyperthermic stress.

D. FACTORS AFFECTING HEAT SENSITIVITY

Exponential Growth

- 2.43 The growth rate of exponentially growing Hela cells was found to be greatest at 37.5°C. falling off steeply with increasing temperature, and being completely inhibited at 41°C. Lag phase cultures, being metabolically active, but not actively dividing, were more resistant than exponentially growing cells (Dickson and Shah, 1972). This was not true for the V79 Chinese hamster cells which showed no difference in sensitivity between cells in either phase (Schulman and Hall, 1974).

Nutritional Status

- 2.44 Chronically hypoxic or nutritionally deprived cells have been shown to be more sensitive to hyperthermia. That this effect is due to nutritional deprivation rather than anoxia has been suggested by Hahn (1976).

Contract Inhibition

- 2.45 Serially propagated growing heteroploid human cancer cells and growing diploid human skin fibroblasts do not survive incubation at

42°C. for 24 hours. Contact inhibited monolayers are still viable and show an increase in thymidine and uridine uptake at 42°C. (Bender and Schramm, 1966). This finding may explain the differential sensitivity of some tumour cells which are rarely contact inhibited. It is probably that the minimal level of DNA synthesis in contact inhibited cells endows them with this measure of protection against elevated temperature (Cavaliere et al, 1967, Levine and Robbins, 1970, Kase and Hahn, 1974).

Method of Transformation

2.47 Cells transformed in vitro by methyl cholanthrene or X irradiation were more heat sensitive than cells transformed in vitro by viral particles (Ossovaski and Sacks, 1967). In contrast tumours produced in vivo by viral transformation were more sensitive to hyperthermia than those induced by physical agents (Kachini and Sabin, 1969).

E. ACQUIRED RESISTANCE OF CELLS TO
HYPERTHERMIA

2.48 As a biological phenomenon, thermal tolerance is usually associated with cells that are genetically endowed for existence at high temperature levels. Thermophilic bacteria either have protein enzymes of unusual heat stability or the rate of heat denaturation of proteins may be counter balanced by an increased rate of synthesis (Adye, Koffler and Mallett, 1967).

2.49 Harris (1967 A.), however, found thermal resistant cells in a seemingly homogeneous clonal line of pig kidney cells following exposure at 47°C. for 90 minutes. The surviving fraction, after recovery at 37°C. showed a decrease rate of heat induced mortality, as compared with the original parental population, when subsequently incubated at 46°C. The clone of heat resistant cells produced were able to pass on this property to daughter cells for several generations without further heat stimulation being required (Harris, 1967 B.). There was no apparent change in the genetic material of such cells but following further exposure to heat they did exhibit a small colony phenomenon, the size of recovering colonies being inversely proportional to the temperature of heat stress.

The Rate of Cell Death Related to Temperature
and Time.

2.50 The death rate of cells undergoing hyper-thermic stress is related to the degree of stress to which they are subjected. Figure 2 shows a typical sigmoidal survival curve for Hela cells (After Palzer and Heidelberger, 1973). Each tissue has its own survival curve for set conditions, which is dependant on its heat sensitivity. This was extensively studied in various tissues of the frog by Orr (1955), a summary of his results is shown in Figure 3. This variation of heat sensitivity is also true for individual animal species. (Figure 4).

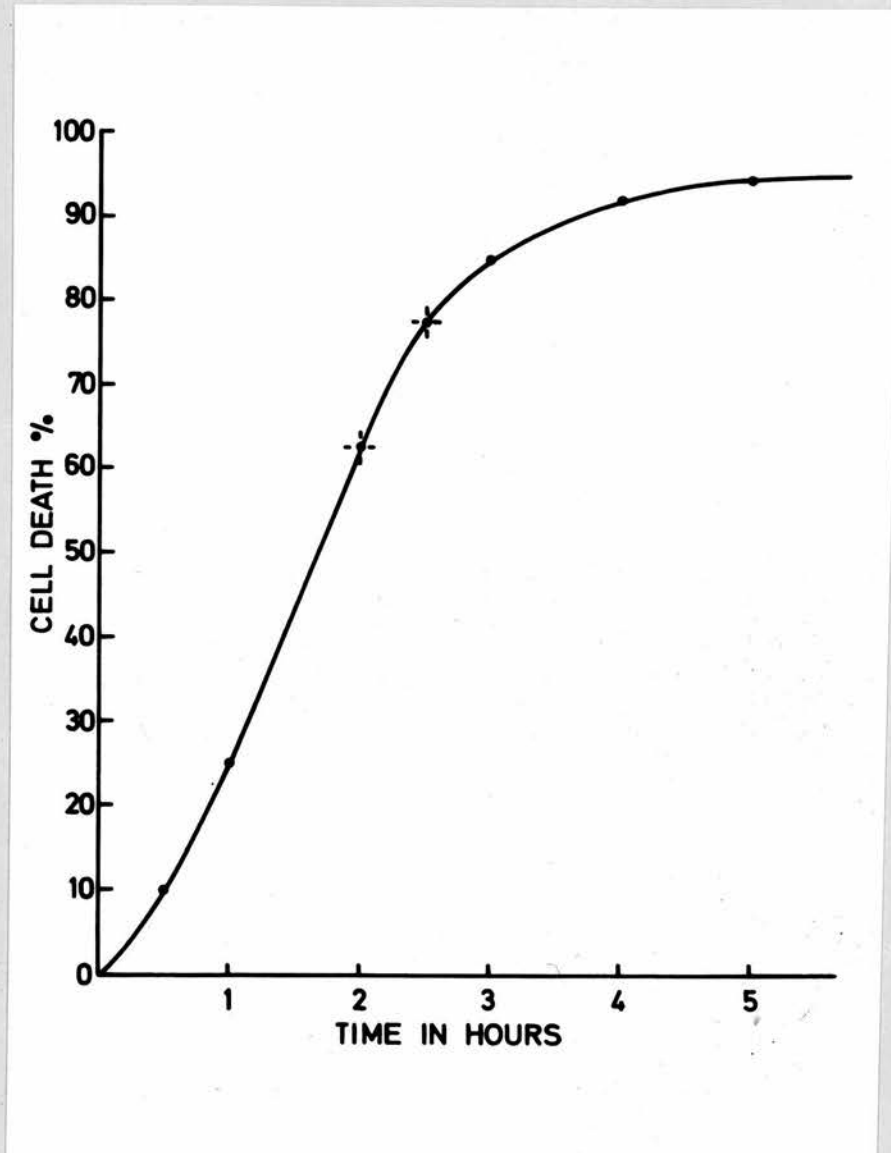


Figure 2.

A typical cell survival curve for Hela cells incubated at 42°C. for various times.
(After Palzer and Heidelberger, 1973).

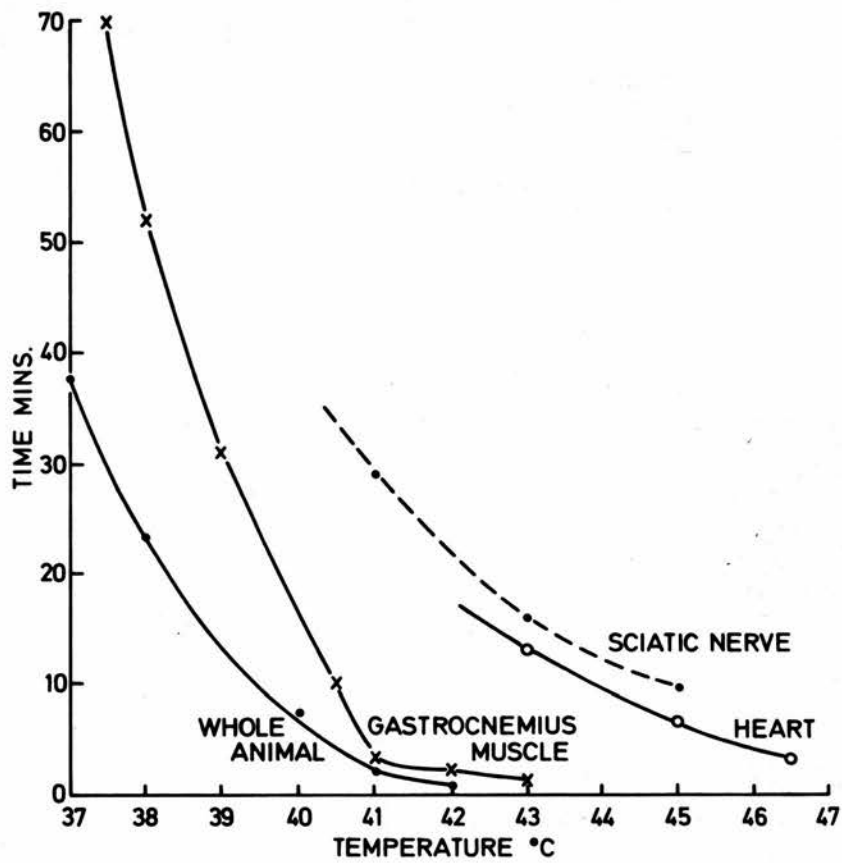


FIGURE 3.

Thermal death rate related to temperature and time for different tissues in *Rana Pipiens*.
(After Orr, 1955.)

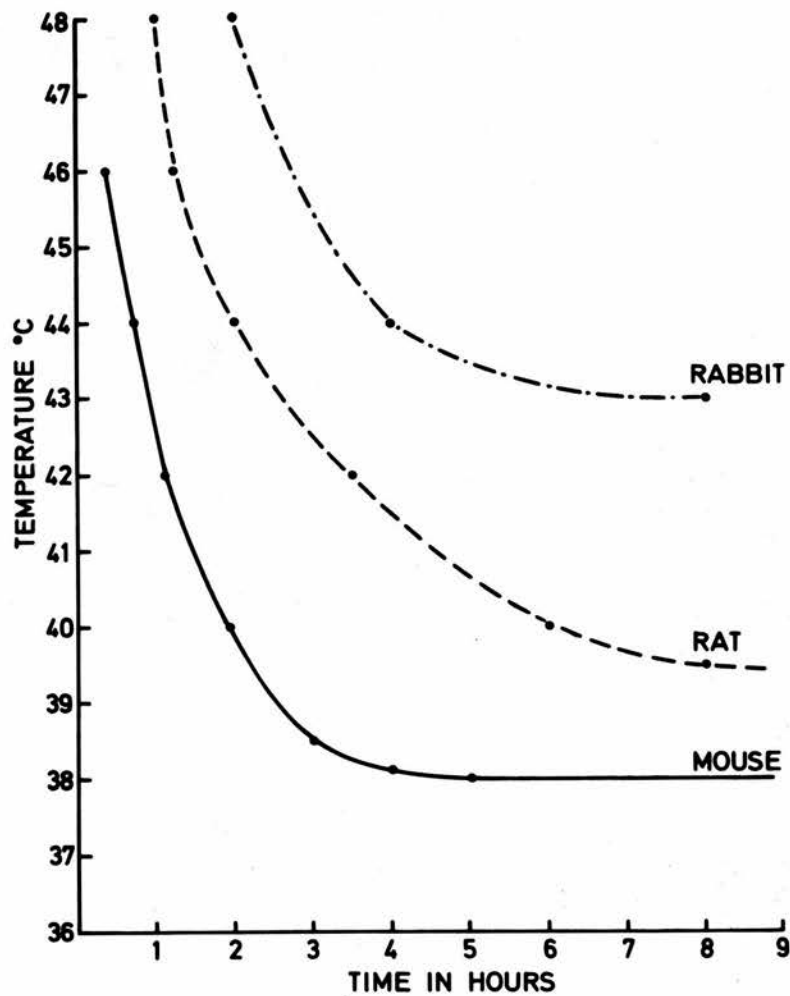


FIGURE 4.

Thermal death rate related to temperature and time for different species.
(After Adolph, 1947.)

F. RATE OF CELL DEATH RELATED TO
TEMPERATURE AND TIME.

- 2.51 In 1971 Westra and Dewey studied the quantitative relationship between rate of cell death at a fixed incubation temperature and the duration that the cells were kept at that temperature. This again shows a sigmoid curve mimicking that of an X-ray dose related survival curve. These results are in good agreement with the formula of Arrhenius for the change in rate of a chemical reaction with temperature.
- 2.52 Arrhenius in 1889 studied the quantitative effect of temperature on the rate of hydrolysis of sucrose and found that it was too great to be accounted for in terms of the effect of temperature on the kinetic energy of the molecule alone. He found that the relationship between the velocity of reaction K_t at one temperature T_o and the velocity K_t at a different temperature T_1 is described by the equation:

$$K_t = K_{t_o} e^{\frac{U}{2} \left(\frac{T_1 - T_o}{T_1 \cdot T_o} \right)}$$

In which U is a constant called the inactivation energy, e is the base of the natural logarithms, T_o and T_1 the absolute temperatures corresponding to T_o and T_1 respectively.

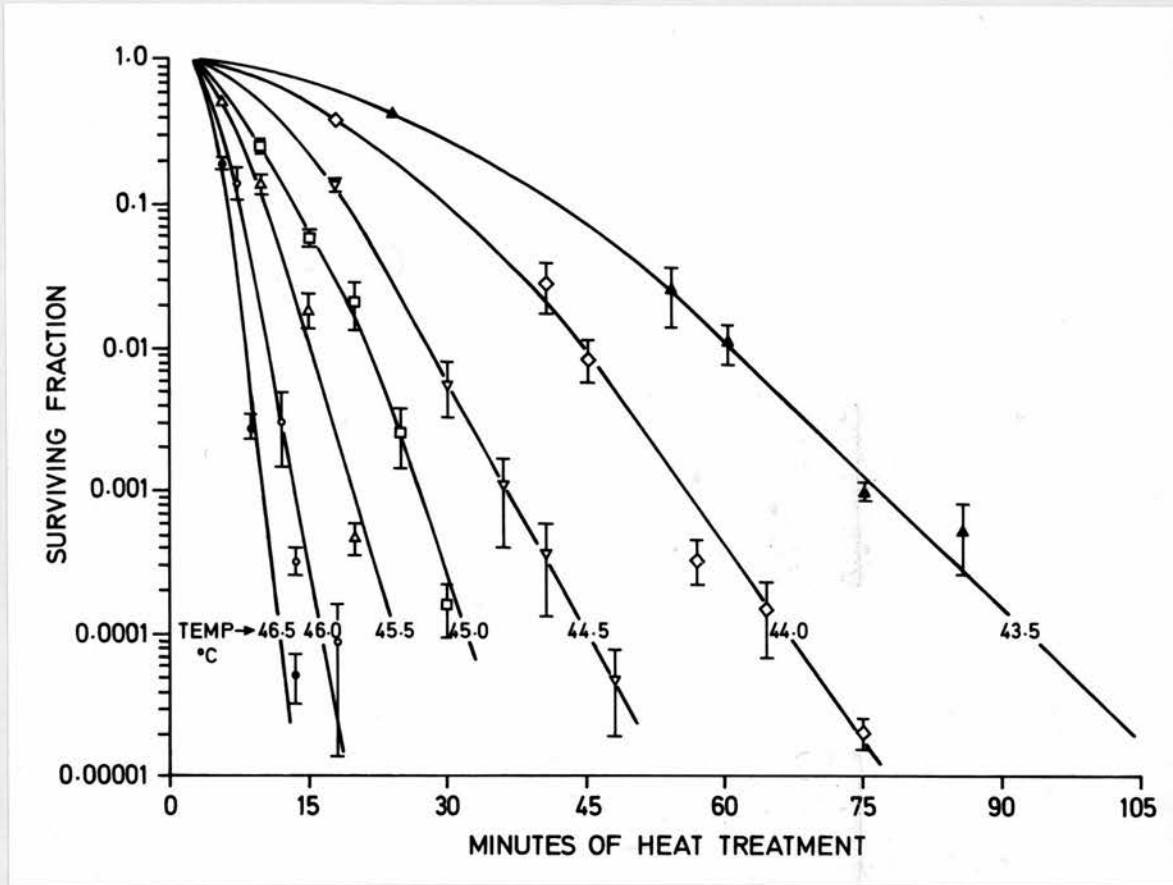


FIGURE 5.

Survival curves for Asynchronous hamster cells incubated at different temperatures as shown. (After Westra and Dewey, 1971).

2.53 The rate of heat inactivation increases by about 12% per degree in an exponential proportion as opposed to the increase in kinetic energy which amounts to only 1/6% per degree rise (Johnson, Eyring and Polissar, 1954). The kinetics of heat inactivation were determined by Westra and Dewey (1971) working with Chinese hamster ovary cells subjecting asynchronous cell populations for different time intervals to temperatures varying between 43.5 and 46.5°C. The resulting survival curves, Figure 5., indicate that the inactivation was sigmoidal in response, i.e. a shoulder followed by a straight line exponential portion.

2.54 The slopes of the exponential portions of the curve gave values for inactivation rates. The inactivation rate is equal to $1/D37$ where D37 is defined as the treatment time required to reduce the survival on the exponential part of the curve to 37% of the initial value. Comparing D37 values for different temperatures showed that for each 1°C rise in temperature the inactivation rate was approximately doubled.

2.55 In order to assess the rate of change in heat sensitivity an Arrhenius type plot was used. In this logarithms of the inactivation rates ($1/D37$) are related to the absolute temperature. This produced a straight line graph (Figure 6.) the slope of which, U is the inactivation energy.

The slope of the graph was approximately the same for Chinese hamster ovary cells and pig kidney cells, with a slope or inactivation energy of 141 K Cals/mole though the sensitivity of the hamster cells was ten times greater. This was reflected by a smaller shoulder on the survival curve with cell death starting at a lower temperature, the rate of change of death rate with increasing temperature was however the same (Suit, 1974).

- 2.56 Some cells, such as Hela cells have a resistant 'tail' at lower temperatures but the overall shape of the survival curve was found to be the same (Palzer and Heidelberger, 1973). Relative values of the slope of the survival curves have been calculated in temperature range for 43 - 46.5°C., they are as follows:-

Jensen Sarcoma in vitro	0.65
Flexner Joblins Carcinoma in vitro	0.5 - 0.8
Crocker Mouse Sarcoma in vitro	0.7
Chinese hamster Cells in vitro	0.5
Pig kidney cells in vitro	0.5
Mouse Feet in vitro	0.5
Sarcoma 180 in vivo	

Below 43°C. there is a varying sensitivity of differing tumours due to the appearance of resistant tail effect (Dewey, Hopwood et al, 1976).

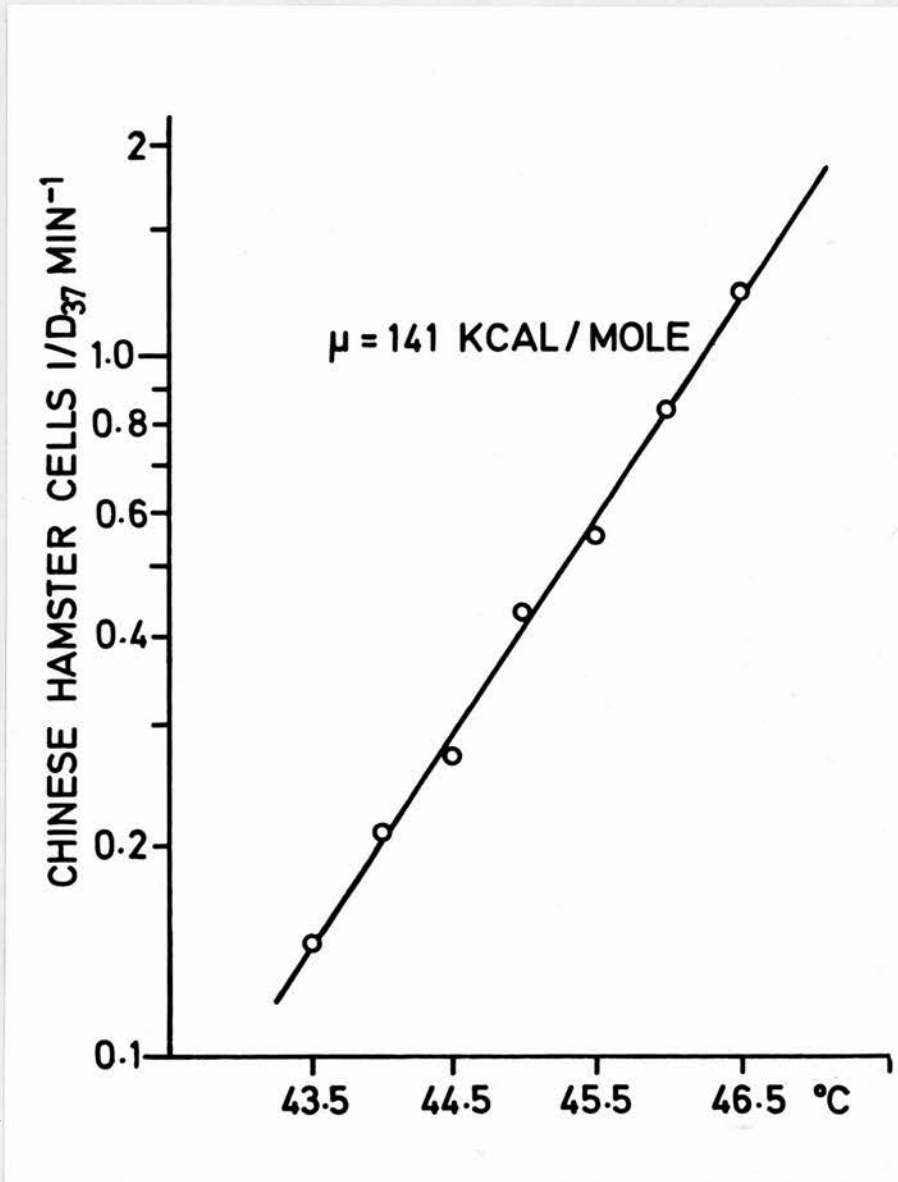


FIGURE 6.

An Arrhenius plot for heat inactivation of Chinese hamster ovary cells. On the ordinate the reciprocal of the D₃₇ values (inactivation rates) are plotted against temperature in °C. The energy of inactivation equals the slope of the graph and is equivalent to 141 K Cals/mole. (After Westra and Dewey, 1971).

G. ANIMAL STUDIES IN VIVOLocalised Heating

2.57 In 1927 Westermarck set out to answer the question, "is it possible to affect a tumour in tissues by heat alone in such a way that there is only tumour damage with sparing of surrounding normal tissue?" He treated rats who had Flexner-Joblings' carcinoma or Jensens sarcoma, by means of high frequency diathermy currents, applied locally and measured tissue temperatures with a thermocouple. He found that the tumour would disappear with 20 minutes at $48^{\circ}\text{C}.$, 30 minutes at $47^{\circ}\text{C}.$, 50 minutes at $46^{\circ}\text{C}.$, 90 minutes at $45^{\circ}\text{C}.$ or 120 minutes at $44^{\circ}\text{C}.$, which is in good agreement with the Arrhenius plot for a constant energy of activation. He found that at $48^{\circ}\text{C}.$ there was no appreciable difference between the time required to kill the tumour and normal tissues but at $44^{\circ}\text{C}.$ the tumour was damaged more quickly by heat than was the surrounding normal tissue.

2.58 Overgaard and Okkels, (1940) treated Woods sarcoma implated subcutaneously in the tails of mice by means of diathermy so raising the tumour to various temperatures and showed tumour regression in a significant number of cases. In 1961 Crile found that T241 sarcomas implanted into the foot pads of C57BL6 mice could be cured by

immersing the paw in a waterbath at various temperatures for differing periods of time. The majority being cured at 43°C . for 70 minutes, but by varying the temperature and time of exposure he was able to confirm the *in vitro* studies that at temperatures above 42°C . the exposure required to obtain the same biological effect can be halved for each 1°C . rise in temperature (Crile, 1962).

- 2.59 In these studies various single fractions were given using differing temperature time equivalents. There were no cures without loss of the foot in the T241 sarcoma series. Heating paws of DBA/1 mice with similarly implanted S91 melanomas, or implanted sarcoma 180 in SWR mice, to 44°C . for 30 to 40 minutes destroyed a high proportion of tumours without damage to the feet in which the tumours were implanted (Crile, 1963). The differential heat sensitivity of tumour over the surrounding normal tissue to localised heating has also been found by Overgaard and Overgaard, (1972), in mouse mammary carcinoma. In general mouse and rat tumours are very sensitive to heat, hamster tumours being relatively resistant and chicken rous sarcoma tumours are more resistant than the surrounding normal tissue (Gerike, Chandra et al, 1971).

Animal Studies by Whole Body Heating

2.60 The thermal sensitivity of animals to elevated temperature varies considerably (Adolf, 1947) Figure 7, 90% of rats heated to 42°C. for one hour die (Dickson, 1975), whereas rabbits can tolerate this degree of hyperthermia (Dickson and Muckle, 1972). Figure 7 shows the survival curves of various animals subjected to hyperthermia.

2.61 Dickson and Ellis, (1974) worked with transplanted Yoshida tumours in rats have indicated that there may be an increased incidence of metastases following whole body hyperthermia. However, only 12 of 250 animals survived hyperthermia and in this small survival group there was an increased incidence of metastases. In the rabbit there was also an increased rate of metastatic disease following hyperthermia but due to technical difficulties it was not possible to maintain a central body temperature of 42°C. in these animals (Dickson and Muckle, 1972).

2.62 In these two studies the state of the survivors and the technical difficulties encountered indicate the need for further studies in heat resistant animals before the results can be accepted. There has been no increase in metastases

formation following local hyperthermia in other animal series reported.

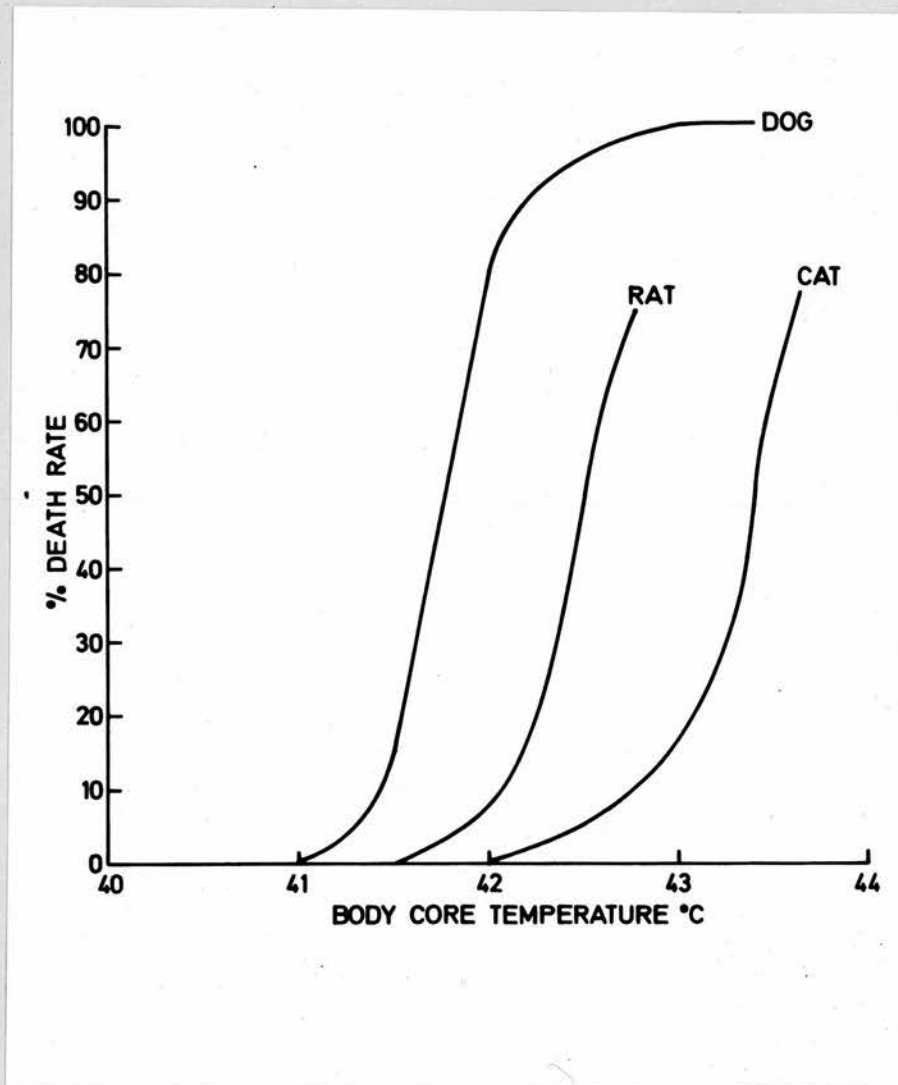


FIGURE 7.

Relative thermal death rates in different species.
(After Adolf, 1947).

SECTION 3.METHODOLOGY OF WHOLE
BODY HYPERTHERMIA.

- A. METHODS OF INDUCING WHOLE BODY HYPERTHERMIA
IN MAN.
- B. SELECTION OF PATIENTS
- C. DR. PETTIGREW'S METHOD OF WHOLE BODY
HYPERTHERMIA.
- D. MATERIALS AND METHODS.
 - THE HAEMODYNAMIC RESPONSE
 - PLASMA, ELECTROLYTE AND ENZYME BALANCE
 - LIVER FUNCTION.
 - PLASMA CORTISOL AND ACTH LEVELS
 - MARROW STUDIES AND CHANGES IN
HAEMOGLOBIN CONCENTRATIONS.
 - WHITE BLOOD COUNT AND THE IMMUNE
RESPONSE.
 - INTRAVASCULAR CO-AGULATION STUDIES.
- E. METHOD OF ASSESSMENT OF CLINICAL RESPONSE
TO HYPERTHERMIA.

A. METHODS OF INDUCING WHOLE BODY
HYPERTHERMIA IN MAN.

3.1. Methods of inducing whole body hyperthermia have been numerous indicating the technical difficulties involved. All methods depend on preventing the physiological process of heat loss from the body and generating an increase in temperature by either increasing the patient's own metabolic rate or applying an external heat source. At temperatures over 40°C. there is a collapse of the normal thermoregulatory systems of the body and it is then necessary to provide an external control system (Stolwijk, 1975).

3.2. The importance of a safe, easily controlled method is stressed by the report from the United States Council of Physical Therapy (1934) which reported 29 deaths in 2,408 patients treated with a lesser degree hyperthermia for venereal disease. Patients with disseminated cancer already seriously ill and any temperature control system must be simple, easy to control, have a high degree of accuracy and allow full access to the patient at all times.

Increase of Internal Heat Production

3.3. The injection of toxins, like Coley's fluid,

or hormones such as Thyroxine, produce a slow rise to 40°C . The effects are unpredictable, especially when given to already very ill patients, and there may be a stimulatory effect on the spread and growth of metastases at temperatures below 40°C . (Dickson and Ellis, 1974). Toxins however, are easy to administer and repeated injections have cured patients with advanced cancer (Nauts, 1946).

External Radiant Heat Sources

3.4

Microwave and ultrasonic sources of energy are the most difficult to control and cannot at present be used for whole body treatment. External sources otherwise rely on the skin conduction. For efficient heat exchange the maximum area exposed for heat uptake is critical as a skin temperature of 45°C . or above for any length of time causes burns (Moritz, 1947). Infrared heating lamps are normally only exposed to 50% of the available area as the couch is not usually heated. These methods also cause a poor exchange rate as the evaporation of sweat cools the skin surface. Patients are enclosed and access is therefore limited. Stafford Warren, (1935) used a box incorporating diathermy in the couch surface, on which the patient lay, with lights in the lid of the box. He treated numerous tumour cases at 41.5°C . for periods of time ranging between four and twenty four hours. He was



treating tumour recurrence following conventional therapy and reported numerous regressions but no cures.

External Heating by Hot Air Currents

- 3.5 Air heating involves enclosing the patient in a constant stream of heated air. The patient must be insulated to prevent evaporation of sweat otherwise excessive skin temperatures are required to cause a net influx of heat energy. The method requires a closed system and the accessibility of the patient is therefore reduced. The air temperature can however be rapidly changed and is easily controlled. The method is being used by Stirling Edwards (1976) to treat patients with advanced cancer. The rate of heating was not as great as immersion techniques but the temperature was easy to control.

External Heating by Immersion in Heated Water.

- 3.6. The immersion of the patient in hot water requires a complicated system for accurate temperature control. As the patient is unable to evaporate sweat due to the surrounding humidity the body core temperature is wholly dependant on the surrounding water temperature. A constant

flow technique is required whereby the temperature of the water can be changed quickly. Circulation of water is also required to avoid conduction difficulties due to local areas of stagnant hot or cold water. Water has the advantages that because of buoyancy there are few pressure areas and the patient is exposed providing easy access during treatment. Water baths have been used by Suryanarayan (1966) to elevate body core temperature for 30 - 40 minutes at $42^{\circ}\text{C}.$, or by Von Ardenne (1969) using a more sophisticated apparatus for longer periods of time at $40^{\circ}\text{C}.$

3.7 In both series cytotoxic drugs were used along with elevation of body temperature for the treatment of disseminated cancer. The results of Von Ardenne's work using a multistep treatment regime, whose 'optimisation' has changed several times during his series are particularly difficult to assess. No cures are claimed in either series though numerous worthwhile remissions have been induced. One disadvantage of this method is the long time, almost two hours, required to elevate the body temperature to $40^{\circ}\text{C}.$

B. SELECTION OF PATIENTS

3.8. Patients in the terminal stages of disseminated cancer were considered for hyperthermic treatment after failure of conventional therapy to control the disease. Treatments considered, including surgery, radiotherapy or cytotoxic chemotherapy, and it was only when no other method of treatment had any likelihood of improving the quality of life or causing tumour regression was hyperthermic therapy offered to the patient. Patients were only treated if they had symptoms such as severe pain and if their life expectancy could be assessed in days or weeks rather than months. No patient referred who fulfilled these criteria was refused treatment.

3.9. No attempt was made to treat any one group of tumours but it was hoped by palliative treatment in this extremely ill group of patients that we would be able to identify the sensitive tumours. We might then be able, at a later date, to offer a curative treatment to fitter patients earlier in the disease.

3.10. All patients knew the nature of their disease, that the treatment was experimental, and that we were not able to say that the treatment would be effective in their case. We did not offer curative treatment but hoped to relieve pain, to improve

the quality of life, and to cause some degree of tumour regression. Before treatment the nature of the treatment and its possible side effects were discussed with the patient and their relatives and fully informed consent to treatment was given by all patients in this study.

- 3.11. Patients were admitted to various wards in the Western General Hospital and treated the following day. They were looked after overnight in the intensive care unit at the Western General Hospital and then returned to their wards. Some patients early in the series, who received the same dosage fraction each week were able to come in on the day of treatment and return home the following day. Later with treatment by increasing dosage fractions, patients had to be kept in hospital between treatment fractions.

C. DR. PETTIGREW'S METHOD OF INDUCING WHOLE BODY HYPERTHERMIA.

- 3.12. The patient was premeditated with Promethazine Hydrochloride, induced with Thiopentone Sodium and curarised. An epidural catheter is introduced at the L4 - 5 level and taped securely in place. The patient is placed in a double envelope of polythene in a specially constructed bath (Figure 8.) Ventilation is

maintained through an insulated endotracheal tube using an oxygen/air/nitrous oxide mixture heated to 80°C . by passage through a steam driven heat exchanger. Using dry air this imparts 6 calories per 500 ml. breath (Moritz, 1945), which offsets the loss of 13 calories per breath due to the latent heat of evaporation of the 23 Grams of water required to saturate 500 mls. of dry air (Moritz, 1945). A urinary catheter and nasogastric tube were inserted and left on continuous free drainage during treatment.

3.13 Three esophageal and three rectal thermometers are placed at 3 cm. intervals to ensure good mucosal contact of at least one thermometer in each group. The highest reading thermometer is taken as the correct reading. A tracheal thermometer and more recently a tympanic probe were also inserted giving a total of eight thermometers which record continuously on two different systems in case of failure of one.

3.14 After heat sealing the polythene envelope surrounding the patient low melting point paraffin wax (melting point $43 - 46^{\circ}\text{C}$. heated to 50°C . is pumped into the bath surrounding the patient. (Figure 9.) The normal physiological process of heat loss is reversed and energy is introduced into the body at a rate of approximately 3,000 calories per minute (Figure 10.) The patient's own

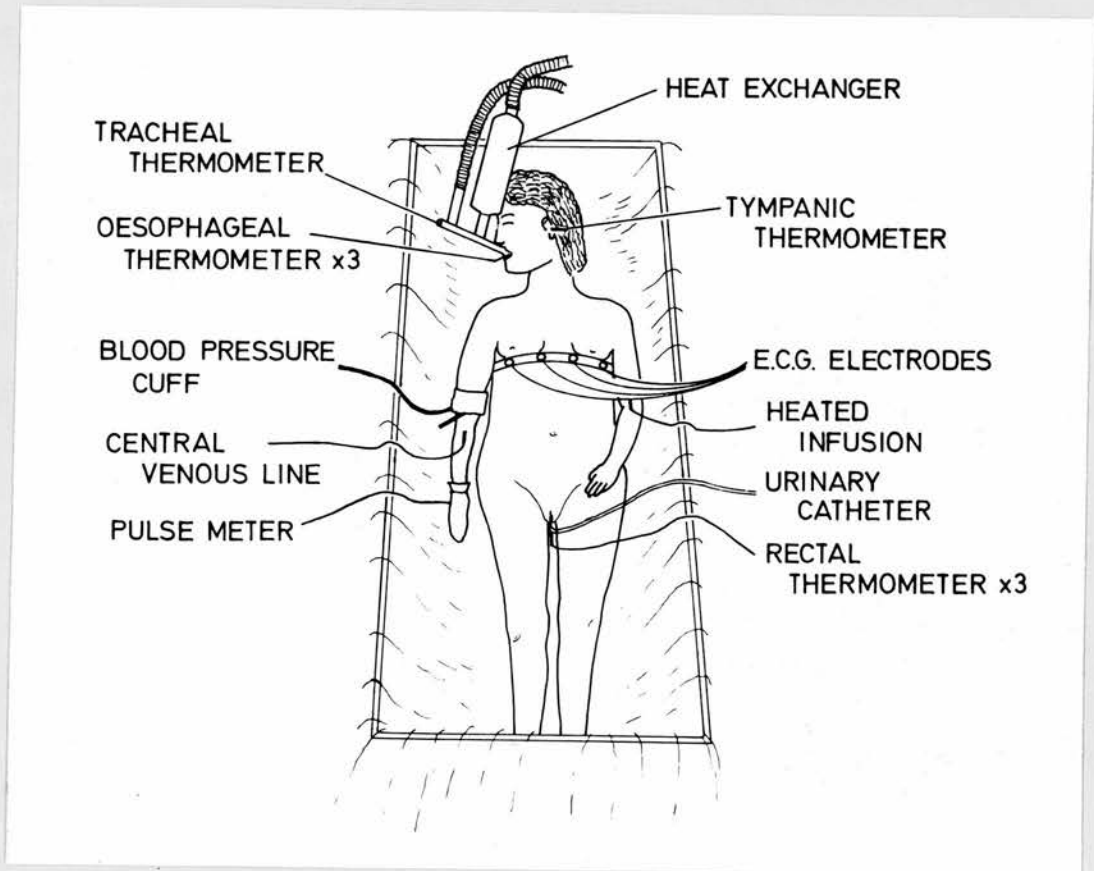


FIGURE 18.

A diagram of the specially constructed bath showing the monitoring devices used during therapy.



FIGURE 9.

Molten wax is pumped into the bath immersing the patient.

SOURCES OF HEAT

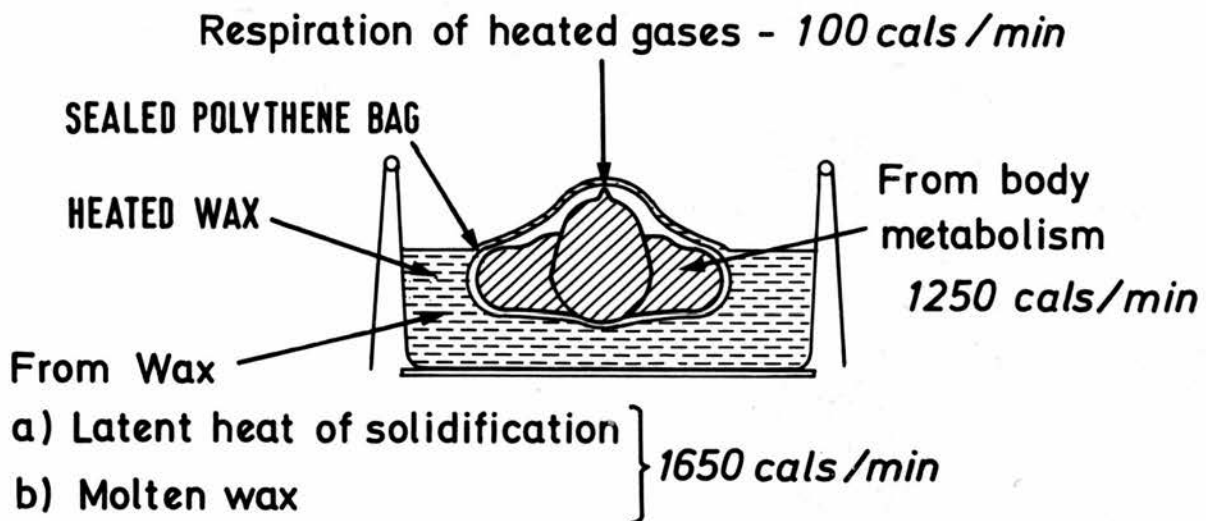


FIGURE 10.

The normal physiological process of heat loss is reversed and energy is absorbed from the sources as shown at a rate of approximately 3,000 calories per minute.

metabolism provides between 1,000 - 1,500 calories per minute increasing by 15% for each 1°C. rise in temperature to 40°C. (Selawry, Carlson and Moore, 1958). Approximately 2,000 calories per minute are absorbed from the wax so raising the body core temperature through 5°C. over a period of approximately one hour.

- 3.15 When the temperature reaches 41°C. all but a thin layer of solid wax is removed, (Figure 11). There follows a slow rise in temperature to 41.8°C. Constant temperatures are then maintained by opening the envelope which encloses the patient and varying the area of skin exposed for the evaporation of sweat (Figure 12.). Narcosis is maintained by intermittent opiates together with a continuous epidural blockade. At the end of the hyperthermia period the patient is removed from the bath and cooling is rapid, (Figure 13.). Care is taken that the cooling does not over-shoot in that the normal thermo-regulatory system takes time to recover and hypothermia could easily occur. The epidural line is left in situ for 24 hours and topped up intermittently for sedation and pain relief.



FIGURE 11.

At a temperature of approximately 41°C . (dependant on body weight), the molten wax is syphoned off, leaving a thin pellicle of solid wax.

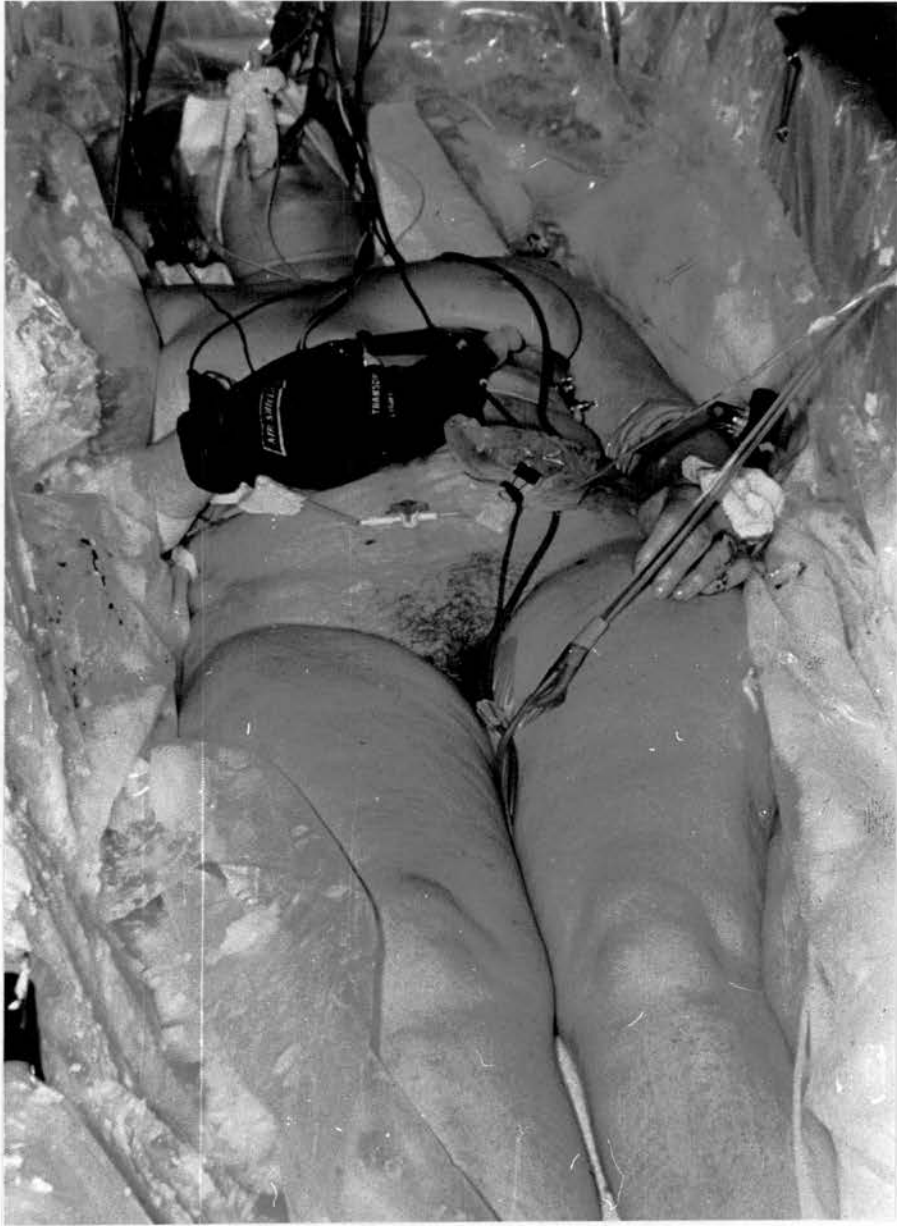


FIGURE 12.

Constant temperature is maintained by opening the envelope which enclosed the patient and varying the area of skin exposed for the evaporation of sweat.

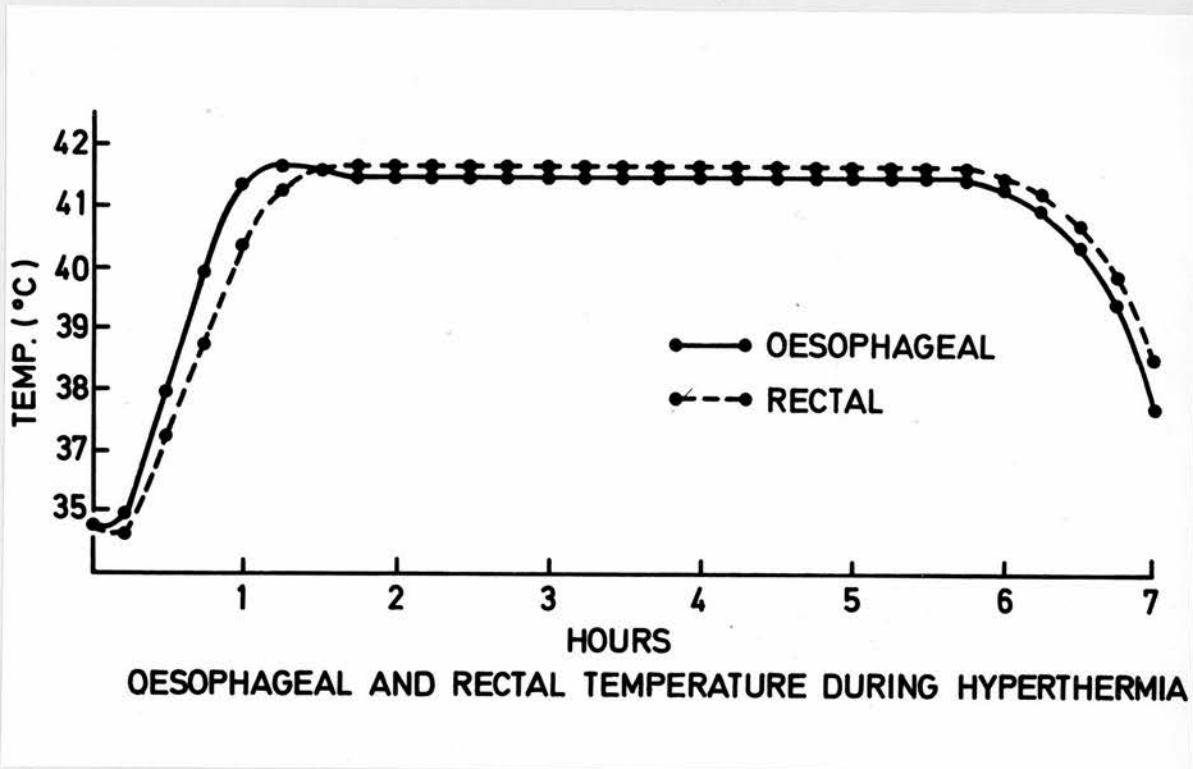


FIGURE 13.

There is a rapid rise to a controlled temperature of 41.8°C. At the end of treatment the patient is removed from the bath and cooling is rapid.

D. MATERIALS AND METHODS

The Haemodynamic Response

3.16 Prior to treatment an 18 guage catheter was introduced into the superior vena cava via the left median cubital vein and was used for measurement of central venous pressure. A second routine intravenous infusion heated to 45°C. by passage through a heating coil, was used for fluid replacement. E.C.G. electrodes were attached in the standard position and a sphygmomanometer cuff attached to the right upper arm. A stethoscope was strapped over the termination of the brachial artery, and a pulse meter working on capillary light transmission attached to the right thumb. Blood pressure was estimated by the use of an arm cuff in the usual way, the pulse meter acting as a check for systolic pressure. Heart rate was measured from the E.C.G. trace which was continuous during treatment. All measurements were taken at 15 minute intervals during treatment.

Plasma, Electrolyte and Enzyme Balance

2.17 Fluid and electrolyte losses were great during hyperthermia and serum electrolyte concentrations, and packed cell volumes were

were measured at half hourly intervals throughout treatment. Results were obtained within 20 minutes of sampling, initially fluids were infused at a rate according to the level of the central venous pressure and its electrolyte constituents varied according to the serum electrolyte concentrations. The packed cell volume was used as a measure of dilution. From these results the intravenous regime recorded in Figure 19 was developed and was used in all subsequent patients. As a check on the accuracy of fluid and salt replacement, losses during hyperthermia were estimated from urinary losses and sweat collection data.

- 3.18 Sweat was assessed by enclosing one limb of each patient in a polythene bag and multiplying the volume of sweat collected in the bag during treatment on the assumption that sweat loss from the limbs and trunk are proportional to their relative surface areas. Data given by Allan, Armstrong and Roddie, (1973) indicates that 60% of the sweat is from the arms and legs, including hands and feet, which together represents 59% of the total body surface area. Half hourly sweat samples were also collected from the trunk on weighed pads of filter paper to measure sweat electrolyte concentrations and variations in sweat rate.

Liver Function; A Limiting Factor in Maximal Temperature.

- 3.19 The serum activities of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase, have been shown to be stable at the temperature involved (42°C.) (Burger, 1970). These were monitored regularly before treatment, and at daily intervals following 64 treatments in 10 patients. A further 10 patients and three controls were studied in depth. In the latter group Bromosulphaphthalein (B.S.P.) tests were carried out prior to induction to anaesthesia, at the end of the hyperthermic period and at between one and seven days post hyperthermia. The three control volunteers were undergoing prolonged anaesthesia for varicose vein operations. Anaesthesia was induced as for the hyperthermia patients though the ventilating gasses were not heated nor was their body core temperature elevated. In this group the B.S.P. tests were carried out before induction of anaesthesia, at the end of the operation and between one and four days later.
- 3.20 The method was used for the B.S.P. test was that an indwelling venous catheter, was inserted and a dose of 3 mg/Kg body weight of B.S.P. for patients undergoing hyperthermia, or 4 mg/Kg body weight for the controls, was injected intravenously into the opposite arm.

Samples of blood were taken immediately prior to injection of B.S.P., at 45 seconds, and subsequently at 2, 7, 10, 15, 20, 25, 35, 45, and 55 minutes.

Plasma Cortisol and ACTH Levels

- 3.21 Serial plasma cortisol levels were estimated in 8 patients during 15 treatment sessions, and serum adrenocorticotrophic hormone (ACTH) in 4 of these patients during 5 treatment sessions. In these patients narcosis was induced prior to hyperthermia by intravenous thiopentone administration (300 - 400 mgs.). Operidine was given intermittently (1 mg. per injection) over a period of 4 - 5 hours. In some instances, valium and/or propranolol were administered, and in one case inotropic drugs were given during a one hour interval, in order to improve cardiovascular function.
- 3.22 Blood samples were taken at regular intervals from the catheter as before, and placed in lithium heparin tubes. These were analysed for plasma cortisol, using a modification of Mattingly (1962) method, within 24 hours of sampling. Similar samples for ACTH estimation were taken and separated promptly, they were then frozen

until the assays were carried out. Plasma ACTH levels were measured by radio immuno assay.

Initially, samples were taken at 20 minute intervals during treatment. In later treatments samples were taken at each centigrade degree rise in the body core temperature and hourly thereafter during treatment. Post treatment samples were taken at 6, 12 and 24 hours.

During treatment the haematocrit was estimated at the same time as the plasma cortisol ACTH concentrations to ensure that no dilution effect occurred.

Marrow Studies and Changes in Haemoglobin Concentration

- 3.23 Sternal marrow punctures were carried out before, immediately after treatment and at one week post treatment. Haemoglobin estimations on blood taken at regular intervals during treatment and daily after treatment and measured by coulter counter. In five patients the osmotic fragility of red blood cells was measured at half hourly intervals during treatment.

White Blood Count and the Immune Response

- 3.24 Blood samples were taken at regular intervals before, during and at daily intervals

following treatment from an indwelling intravenous catheter, as before. White counts were carried out using a coulter counter and a differential count was then done visually.

Serum immunoglobulin levels were measured, before, immediately after, and at ten days post treatment. Blood samples were also taken prior to therapy and at fourteen days for viral studies which consisted of antibody titres to myxovirus influenza B, mycoplasma pneumonia and cystomegalo virus.

Intravascular Co-agulation Studies

3.25 Prior to treatment an intravenous cannula was inserted into the superior vena cava as before. Blood was taken at regular intervals from this cannula before, during and after treatment and estimations carried out for haemoglobin, platelets, prothrombin time, fibrinogen, and fibrinogen degradation products. Three patients received controlled low dose heparin throughout hyperthermia period. In order to standardise the treatment fractions for different temperature time equivalents, the degree minute, 41°C . standard, was taken as the unit of hyperthermic therapy. This was calculated by the temperature in degrees centigrade multiplied by the

time in minutes that the body core temperature was above 40°C . Most patients were treated by serially increasing fractions at weekly intervals though some early cases were not managed in this way. The fraction of treatment received was then plotted against the subsequent changes in platelet count for patients showing a clinical response to therapy as compared with those who did not.

E. METHOD OF ASSESSMENT OF CLINICAL
RESPONSE TO HYPERTHERMIA.

3.26 Patients were assessed prior to treatment at a special clinic which was set up for this purpose. All available information concerning weight loss, tumour size and previous investigations were entered on the specially constructed assessment sheet (Figure 14). Routine X-rays of chest and pelvis were taken and in later cases, liver and spleen scans were routinely carried out. Other X-rays were arranged as indicated. All patients had an E.C.G. prior to treatment. Routine blood was taken for the tests as shown on the review sheet. All documents relating to the patient were examined and the histological slides reviewed by Dr. N. McLean. Coagulation studies were also performed on all patients prior to therapy.

Review was repeated at one week post treatment and monthly subsequent to completion of the prescribed course of hyperthermic fractions.

3.27 The response to treatment was judged favourable if there was weight gain, increased mobility, or pain relief together with objective evidence of regression in tumour size on direct measurement, pathological evidence of necrosis and serial biopsy specimens, or radiological evidence of regression. Further evidence of heat induced tumour necrosis was obtained at necropsy in five of the six patients who died soon after hyperthermia.

3.28 Initially all patients were treated for approximately four hours above 41°C . at weekly intervals. Later patients were fractionated over three treatments of increasing duration.

HYPERTHERMIA REVIEW

NAME

AGE

DATE

DATE LAST TREATMENT

WEIGHT

CYTOTOXICS

OTHER DRUGS

Tumour Size (Mark Area On Figure)

A:

B:

C:

D:

Hepatomegally

R Mid Clav:

Mid Line:

L Mid Clav:

Palpable Fixed

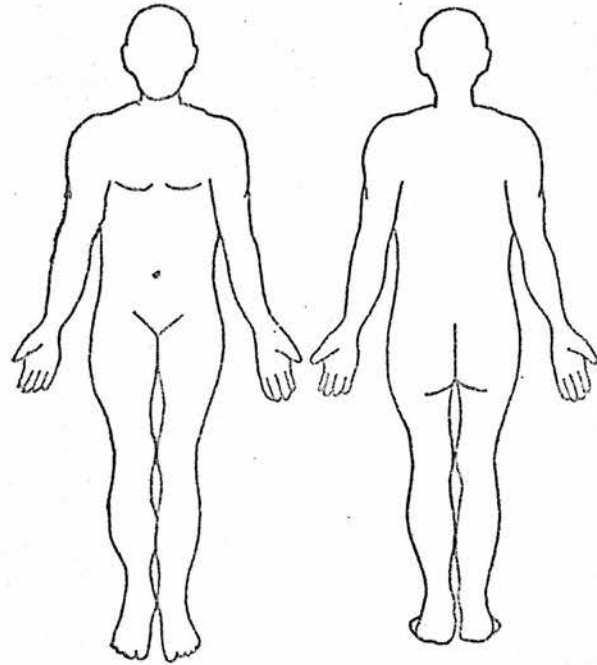
Cervical Nodes

Axillary

Inguinal

Purpura

Jaundice



Mobility - Confined to bed

Ambulant

Walking

Working

Appetite - Increased

Same

Decreased

Pain - Better

Same

Worse

Complications

Investigations

FBC

E+U

Platelets

LFT

Diff WBC

LDH

Retics

SGOT

PTA

CPK

Uric Acid

X-Ray (Chest & Pelvis)

Other Test

Disposal

FIGURE 14.

Hyperthermia review sheet which was completed prior to each treatment and at montly intervals post treatment.

SECTION 4.RESULTS OF WHOLE BODY
HYPERTHERMIA.

- A. THE HAEMODYNAMIC RESPONSE.
 - HEART RATE.
 - BLOOD AND CENTRAL VENOUS PRESSURES.
- B. FLUID AND ELECTROLYTE BALANCE.
- C. LIVER FUNCTION. A LIMITING FACTOR IN
MAXIMAL TEMPERATURES.
- D. THE STRESS RESPONSE; PLASMA CORTISOL
AND ACTH LEVELS.
- E. MARROW STUDIES AND CHANGES IN
HAEMOGLOBIN CONCENTRATION.
- F. WHITE CELL COUNT AND THE IMMUNE RESPONSE.
- G. INTRAVASCULAR COAGULATION; A LIMITING
FACTOR IN FRACTIONATION.
- H. CLINICAL RESULTS OF WHOLE BODY HYPERTHERMIA.
- I. COMPLICATIONS OF WHOLE BODY HYPERTHERMIA.

A. THE HAEMODYNAMIC RESPONSE

Heart Rate

- 4.1. In patients treated without epidural blockade the heart rate increases rapidly with temperature (Figure 15). The increase is linear and averages with 8.5 beats/min./Centigrade degree rise in temperature, with a range of 5 - 15 beats/min./Centigrade degree. In the same patient heated on different occasions the rate of increase in heart rate depends on the rate of heating (Figure 16). Fast heating initially increases the heart rate less per degree rise in temperature. However, at the end of active heating heart rate continues to rise for sometime reaching a typical maximum for each patient at 41.8°C . which is independent of the rate of heating. The final heart rate at stable temperature corresponds to a rise of 11 beats/min./Centigrade degree rise.
- 4.2 Stable narcosis was required to maintain this constant heart rate and an increase in heart rate was found to be one of the first indications of decreasing depth of narcosis. With the high level epidural anaesthesia now used there is a blockade of the sympathetic outflow and the rise in heart rate is less averaging with a range of 7-8.5, beats/min./Centigrade degree rise in temperature (Figure 17). An increase in

heart rate in those patients is indicative of a decrease in the effect of the epidural blockade.

Blood and Central Venous Pressures

4.3 Drugs used in the induction of anaesthesia induce an initial mild degree of hypotension (Figure 18). Thereafter during active heating the systolic blood pressure rises by 20 - 50 mms. of mercury with little change in the diastolic pressure. During active heating there is also a rise in the central venous pressure averaging 5 - 10 centimetres of water. At stable temperatures, these values return to the initial values and tend to fall further unless a plasma expander is infused. For this reason, plasma is given once 41.8°C . is reached (Figure 19). The blood and central venous pressures are thus maintained at the pretreatment value throughout treatment.

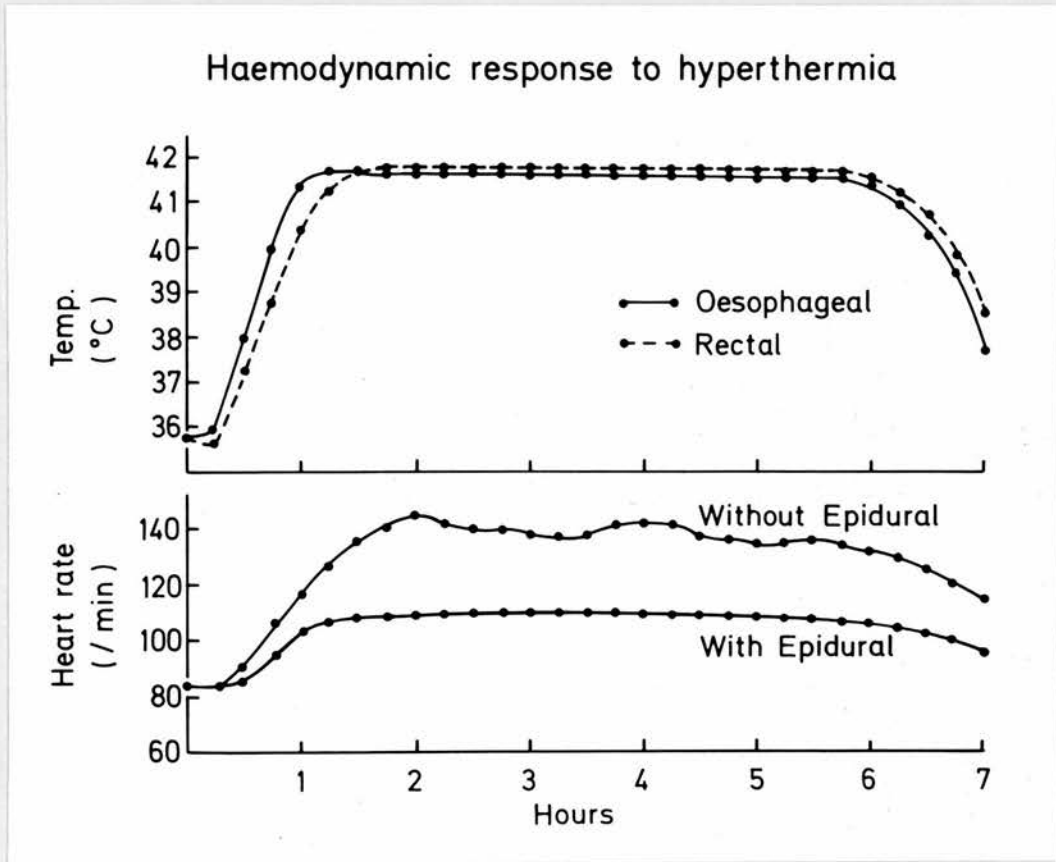


FIGURE 15.

The heart rate increases rapidly with temperature without epidural anaesthesia, stable narcosis was required for control of heart rate. With epidural anaesthesia, the rise in heart rate is less and the variations in rate more easily controlled.

Variation of heart rate with oesophageal temperature during active heating without epidural anaesthesia

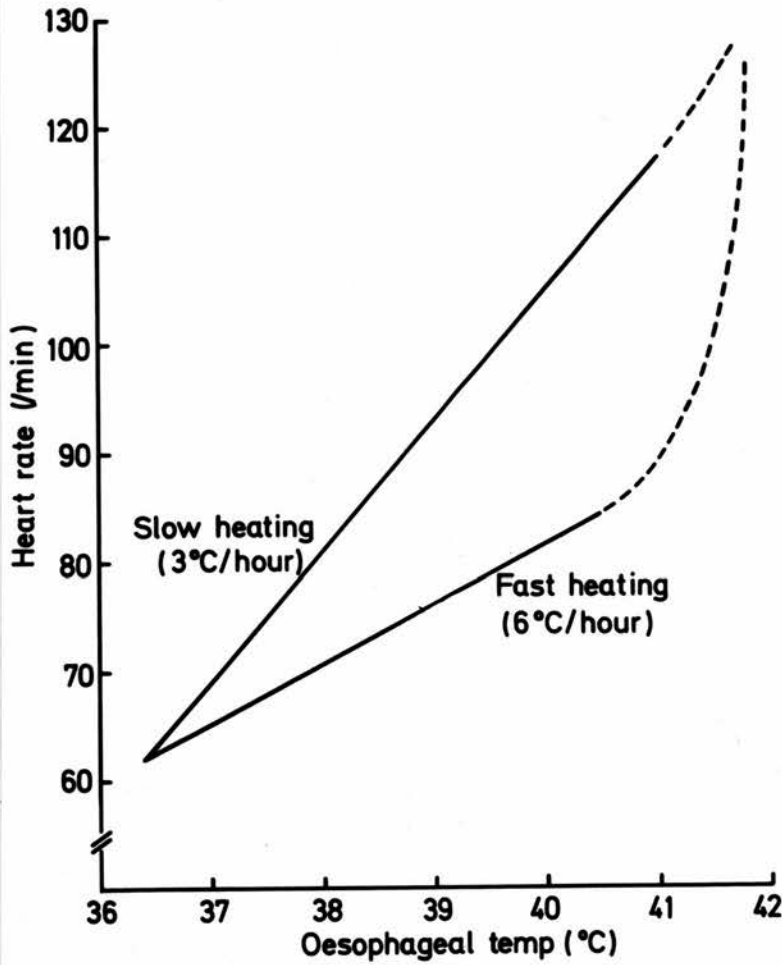


FIGURE 16.

Without epidural blockade there was considerable variations in the heart rate, dependant on the rate of heating and the depth of anaesthesia.

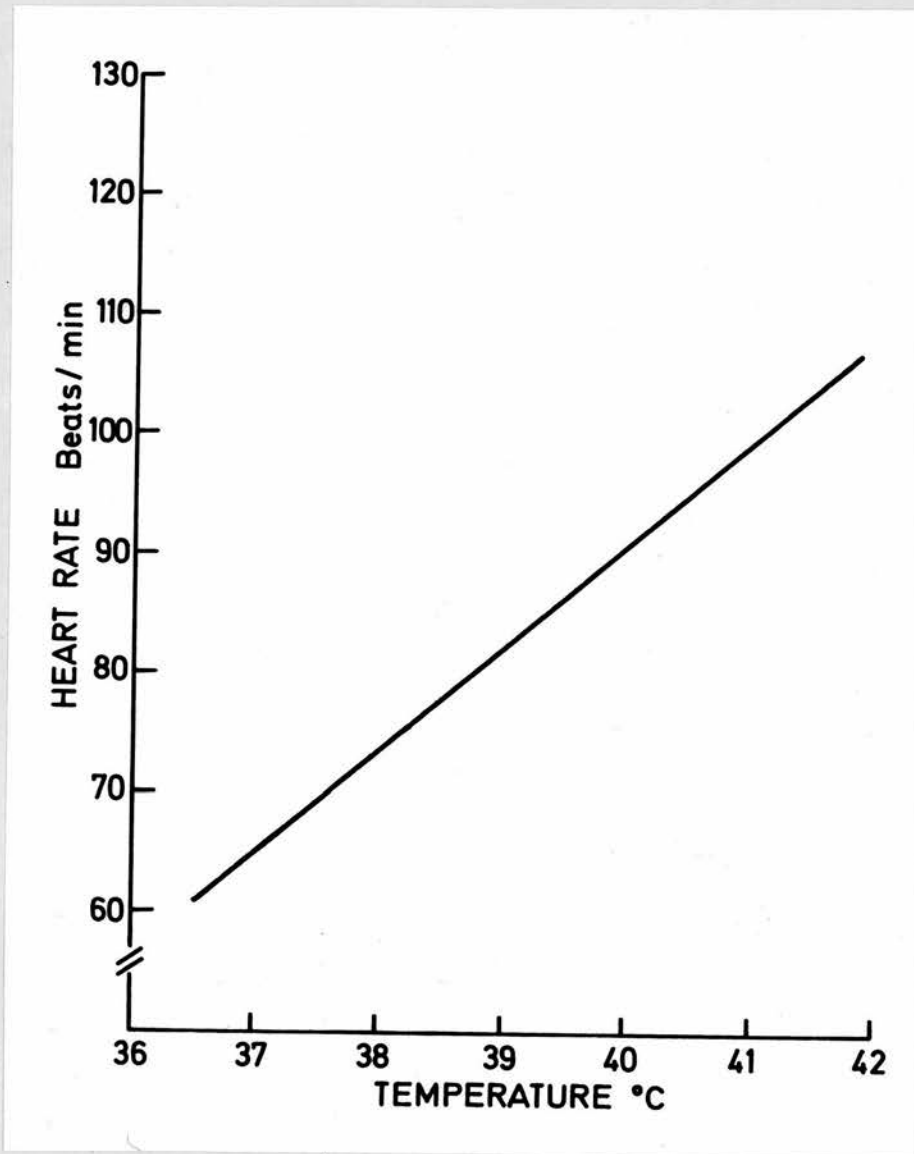


FIGURE 17.

With epidural blockade the heartrate rose linearly with temperature and did not vary with different rates of heating.

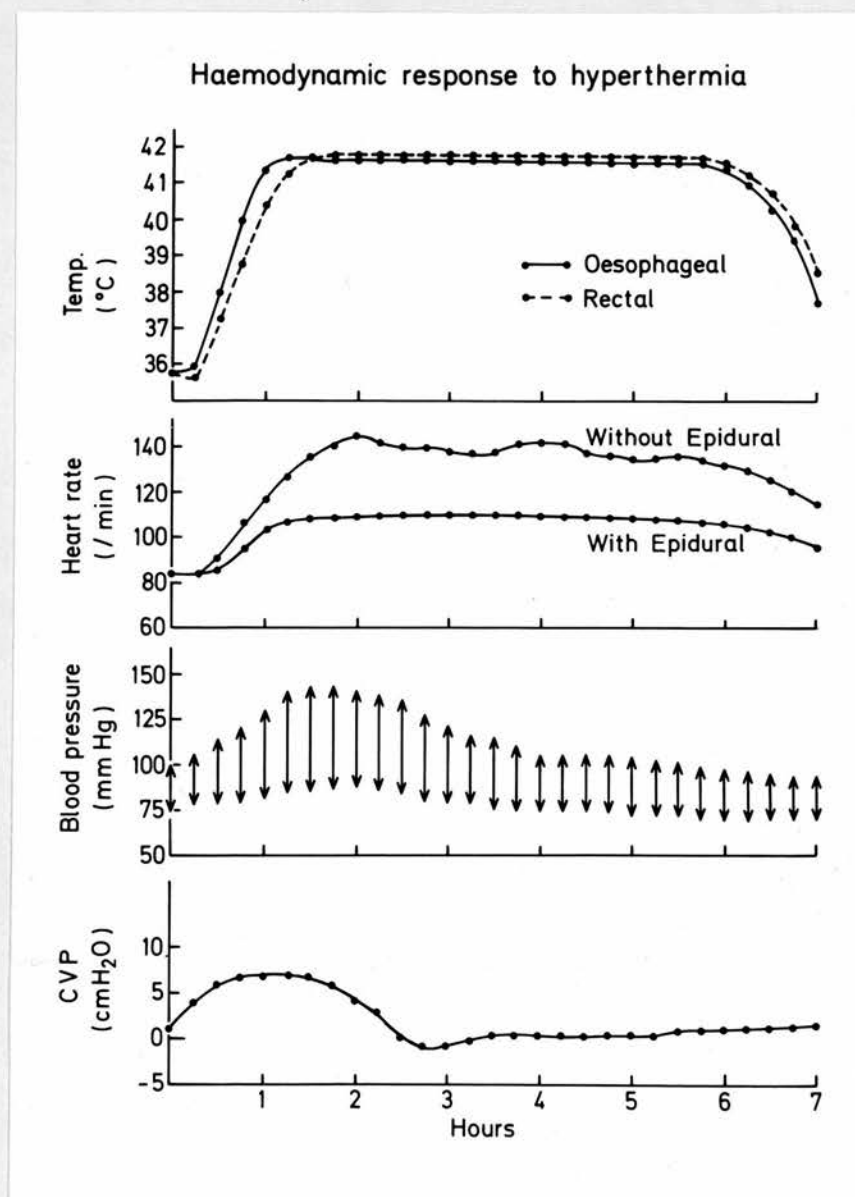
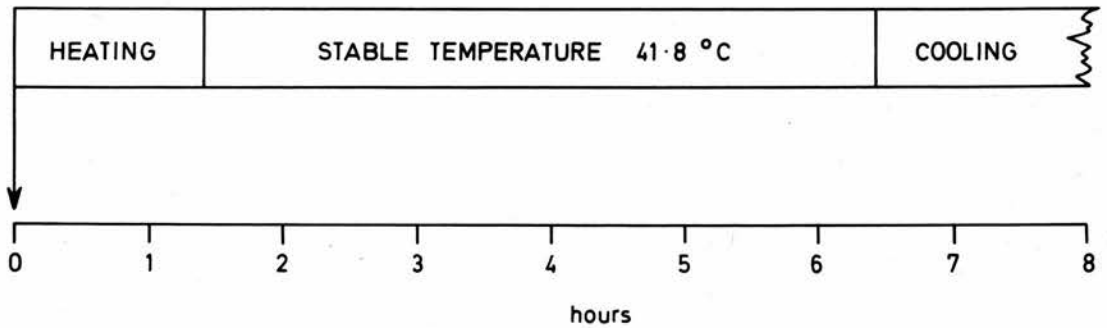


FIGURE 18

A summary chart relating heart rate, blood pressure and central venous pressure to temperature over the treatment session. After an initial rise in blood and central venous pressure there is a fall at stable temperature which can be compensated by the infusion of plasma.

Fluid replacement regime

saline 0.9%	dextrose 5%	dextrose 4.3%	Hartmann's solution	plasma	Hartmann's solution	5% laevulose	Hartmann's solution	Hartmann's solution 4 hourly
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FIGURE 19.

The fluid replacement regime necessary to maintain plasma electrolyte concentrations within the normal range was worked out empirically. Plasma is given at stable temperature to prevent a fall in blood pressure.

B. FLUID AND ELECTROLYTE BALANCE

4.4 With the present infusion regime there was no significant change in the serum sodium or chloride concentrations of the ten patients studied in depth, (Figure 20.). There is a slight early decrease in the serum sodium concentration averaging 4 m.mol/l which coincides with, and has been attributed to premedication (Stevenson, 1960). In 50 treatments on 8 patients the serum sodium and chloride measured 24 hours after treatment showed no significant change from the pre-treatment value. The serum potassium rises by 0.5 m. eq/l. during the first ninety minutes of treatment. This increase is not maintained beyond three hours when a slow fall in potassium concentration starts, and 24 hours after treatment the serum potassium concentration is on average 0.5 m.mol/l lower than the pre-treatment values. This lower level continues for three to four days and occasionally potassium supplements were given.

Sweat and Urinary Losses

4.5 Sweating was profuse during hyperthermia averaging 500 mls/hr, (Figure 21.). The sweat rate increases rapidly with temperature. At

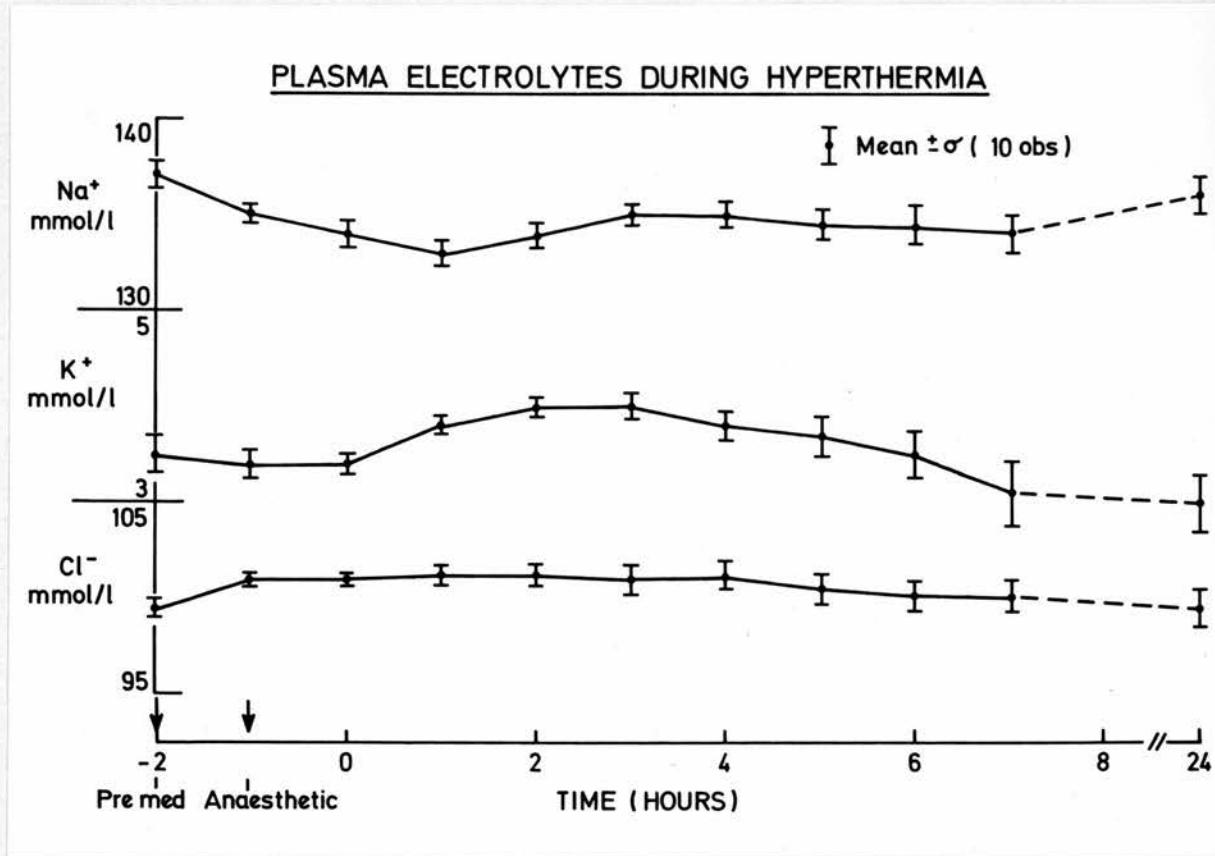


FIGURE 20.

Using the replacement regime there were no changes in the plasma electrolyte concentrations outwith normal limits.

ELECTROLYTE AND FLUID LOSS AND REPLACEMENT DURING HYPERTHERMIA

	ml/hr	mmol/hr			mmol/l			Na:K
		Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻	
Urine*	52 $\sigma=45\%$	3.3 $\sigma=40\%$	2.1 $\sigma=50\%$	3.7 $\sigma=40\%$	67 $\sigma=30\%$	51 $\sigma=25\%$	83 $\sigma=35\%$	1.3:1
Sweat**	514 $\sigma=30\%$	43 $\sigma=40\%$	2.0 $\sigma=20\%$	41 $\sigma=25\%$	84 $\sigma=12\%$	3.6 $\sigma=5\%$	80 $\sigma=12\%$	23:1
Infusion	570	52	1.0	43	-	-	-	-

* mean of 40 measurements

** mean of 60 measurements

FIGURE 21.

Fluid and electrolyte losses were great during hyperthermia but were well balanced by the infusion regime.

stable temperature fluctuations of over 10% occur in the sweat rate; no further increase with continued exposure to high temperatures has been determined. In general patients with a high sweat loss had a low urinary output and vice versa but it was not possible to correlate these differences with any measureable parameter.

C. LIVER FUNCTION; A LIMITING FACTOR
IN MAXIMAL TEMPERATURE.

4.6. In 47 treatments conducted in 10 patients during which neither the rectal nor the oesophageal temperatures rose above 41.8°C . there was no significant increase in the serum enzyme activities and the serum bilirubin concentration rose from an average of 8.0 u mol/l to an average of 10.6 u mol/l . No values lying outside the reference range (Figure 22.). However, during 17 patients within the same group when the temperature was held at between 41.8 and 42.2°C . for periods of between 10 and 40 minutes the average activity of amino-transferase increased by a factor of 25, that of the alanine aminotransferase by a factor of 8 and the serum bilirubin to 26.7 u mol/l . These changes were greatest at 48 hours post treatment, most returning to the pre treatment values within five days.

**SERUM ENZYME AND BILIRUBIN CONCENTRATIONS BEFORE AND AFTER
HYPETHERMIA AT VARIOUS BODY CORE TEMPERATURES**

	REFERENCE RANGE S.I.Units/l	BEFORE HEATING	AFTER HEATING BELOW 41.8 °C	AFTER HEATING ABOVE 41.8 °C
LACTATE DEHYDROGENASE	72-395	270	266	815
ASPARTATE AMINOTRANSFERASE	9-43	29	32	678
ALANINE AMINOTRANSFERASE	5-41	21	25	166
BILIRUBIN	3-14 μmol/l	8.0	10.6	26.7

FIGURE 22.

At body core temperature below 41.8°C. there was no significant changes in serum enzymes or bilirubin concentrations.

4.7 13 Patients were studied in depth and had serum enzymes and B.S.P. tests carried out (Figure 23). These patients were divided into four groups. The anaesthetic controls, those in whom the temperature did not rise above $41.8^{\circ}\text{C}.$, those whose temperature rose above $42^{\circ}\text{C}.$, and the patients with hepatic metastases. Each group was small containing three patients with four patients in the group treated with hepatic metastases. The control group showed no significant changes in liver function or B.S.P. clearance due to the anaesthesia used (Figure 23). All patients treated by hyperthermia had an increased B.S.P. retention at the end of the hyperthermic period, this was greatest in patients treated at over $42^{\circ}\text{C}.$ and in one of these cases, and two of the patients with hepatic metastases, the B.S.P. excretion did not return to the pre-treatment value within 7 days. In all other cases liver function had returned to pre treatment value within 7 days

**LIVER FUNCTION AND BROMSULPHTHALEIN EXCRETION OF 13 PATIENTS BEFORE, DURING
AND AFTER HYPERTHERMIA**

SERUM	NORMAL RANGE	CONTROLS			< 41.8°C			> 41.8°C			LIVER METASTASES		
		PRE	AT	POST	PRE	AT	POST	PRE	AT	POST	PRE	AT	POST
BILIRUBIN	3-14 µmol/l	12.0	10.3	10.3	5.2	5.2	6.8	6.8	37.6	17.1	38.5	47.9	47.9
ALKALINE PHOSPHATASE	20-28 iu/l	50.6	40.3	48.0	120	116	135	83	87	68.5	323	275	285
ASPARTATE AMINO- TRANSFERASE	9-43 u/l	22.7	22.0	24.4	34.2	28.5	34.2	18.5	86.8	18.3	44.6	71.5	85.4
BROMSULPHTHALEIN RETENTION AT 45 mins	< 5 %	1.3	3.6	1.0	4.6	19.3	7.5	10.3	26.7	15	14.8	26.5	18.6

FIGURE 23

Patients studied in depth showed that although there was no rise in serum enzymes following hyperthermia at less than 41.8°C. there was delay in Bromsulphthalein excretion. This was greatest in patients treated at over 41.8°C. and those with liver metastases.

D. THE STRESS RESPONSE; PLASMA
CORTISOL AND ACTH LEVELS.

4.8 The plasma cortisol response to hyperthermia is divided into three phases. Initially, during active heating there is a fall followed by a second phase during which there is a sharp rise returning to the previous values. If heat stress continues over two hours at 41.8°C . there is a further rise. Figure 24 shows the typical response in one patient treated over an extended period and the relationship of body core temperature to plasma ACTH and cortisol levels is shown. During the induction of anaesthesia there is a slight rise in plasma cortisol which then begins to fall as active heating commences. This fall is most marked at temperatures over 38°C . and continues for approximately 1 hour. Figure 25 shows the relationship of plasma ACTH to plasma cortisol during active heating. At a temperature of approximately 38°C . there is a sudden fall in the ACTH level, which then begins to rise sharply reaching the pre treatment level at approximately 39.5°C . at which time the plasma cortisol level is at its lowest. A further rise in ACTH precedes the subsequent rise in cortisol level. A subsequent fall in ACTH is reflected by stabilisation of the cortisol but with increase in temperature there is a further rise in both plasma ACTH and cortisol levels.

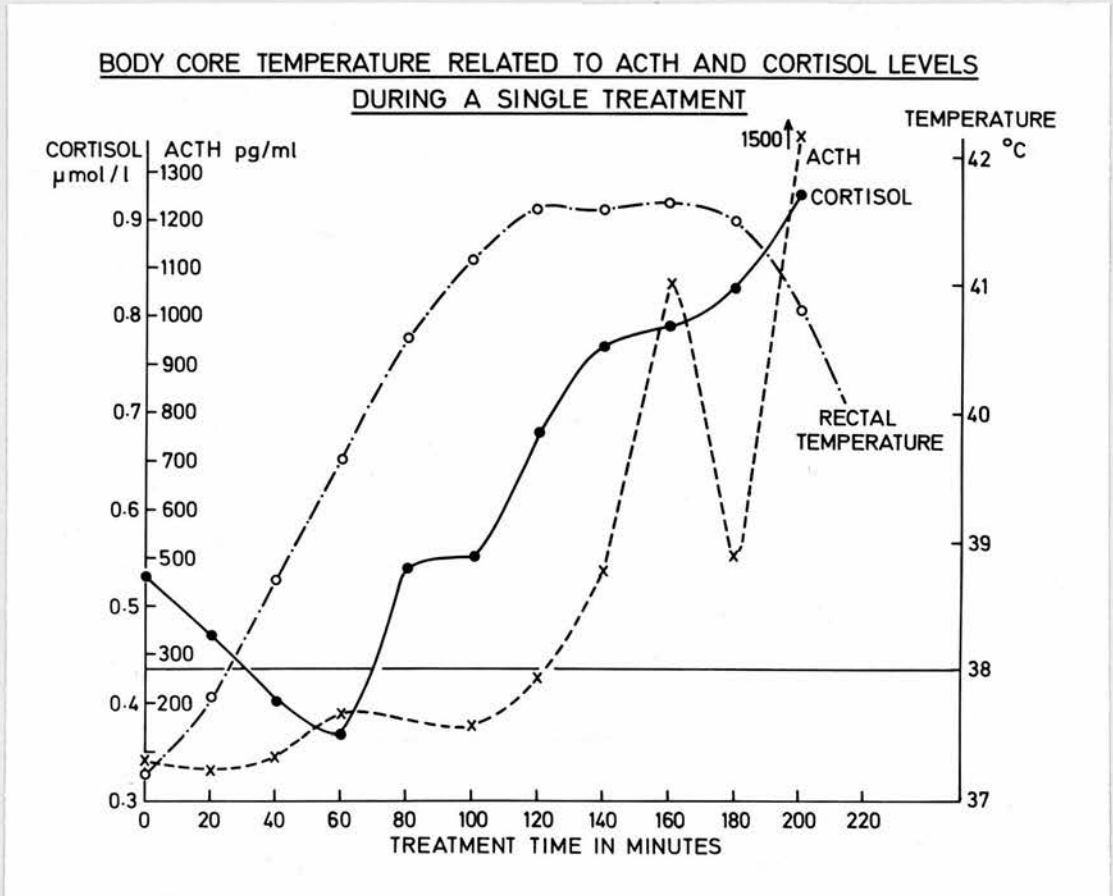


FIGURE 24.

This is a representative figure taken from one patient and shows the initial fall in plasma cortisol followed by a sharp rise in both plasma ACTH and cortisol levels. After prolonged hyperthermia there is a further sharp rise in both levels indicative of continuing thermal stress.

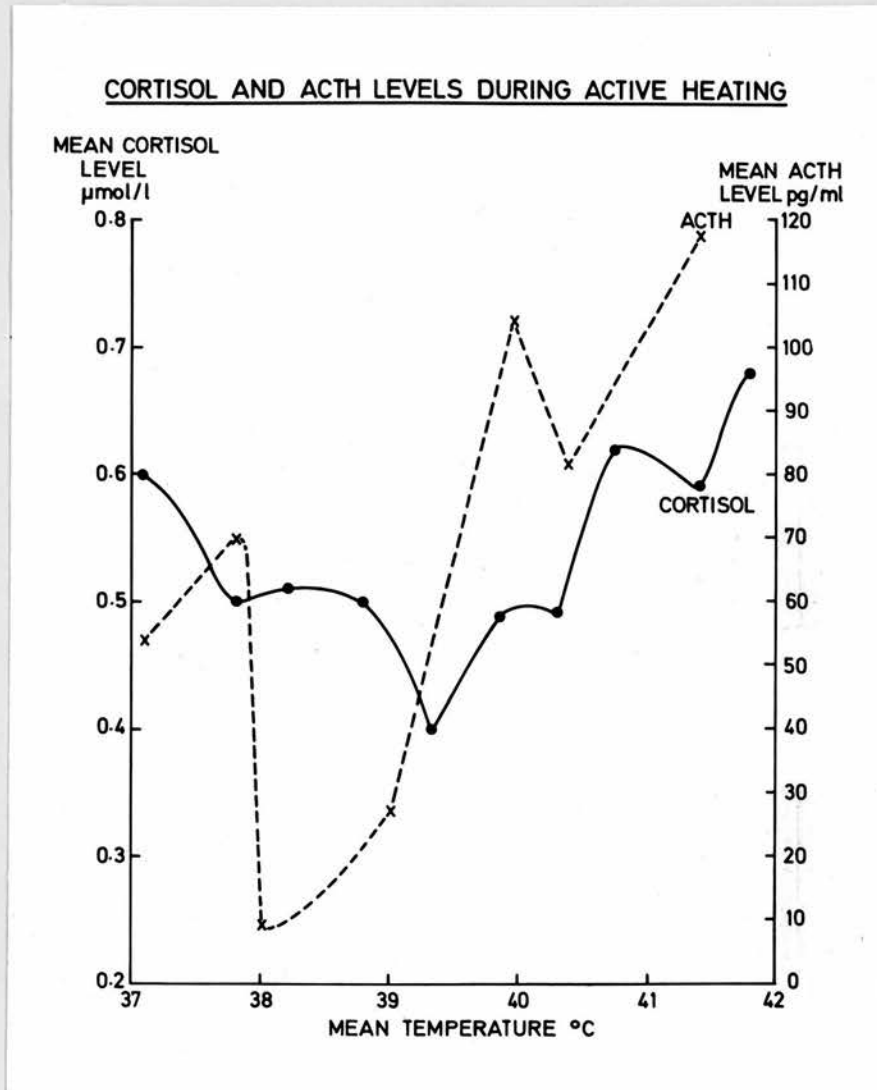


FIGURE 25.

Average ACTH and cortisol levels during active heating shows a fall in both ACTH and cortisol levels followed by a sharp rise.

4.9 To study further the effects of continuing heat stress on adrenocortisol function the mean levels of plasma ACTH and cortisol were plotted against the degree minutes 39°C . standard of heat stress, (Figure 26). This unit was chosen as ACTH inhibition occurred above 38°C . and because in other heat sensitive systems the degree of heat stress is related to temperature and the time that tissue is exposed to that temperature. For each centigrade degree rise in temperature the time taken to achieve the same degree of stress is halved. The centigrade degree minute, 39°C . standard, is equivalent to the time in minutes spent at 39°C . and represents the area under the temperature graph shown in Figure 24. above 38°C .

4.10 There is a rise in plasma cortisol from the lowest point occurring at 25°C minutes, 39°C . standard to pre treatment values. At above 150°C minutes there is a further rapid rise in the ACTH and cortisol levels, (Figure 26), which is independent of the rate of heating. If heat stress is continued beyond 450°C minutes, 39°C . standard, there is a further increase in plasma cortisol and ACTH levels. If the treatment fraction was less than 450°C minutes, 39°C . standard, approximately equivalent to two hours at 41.8°C ., the plasma cortisol returned to the pre treatment value within 8 hours. If the

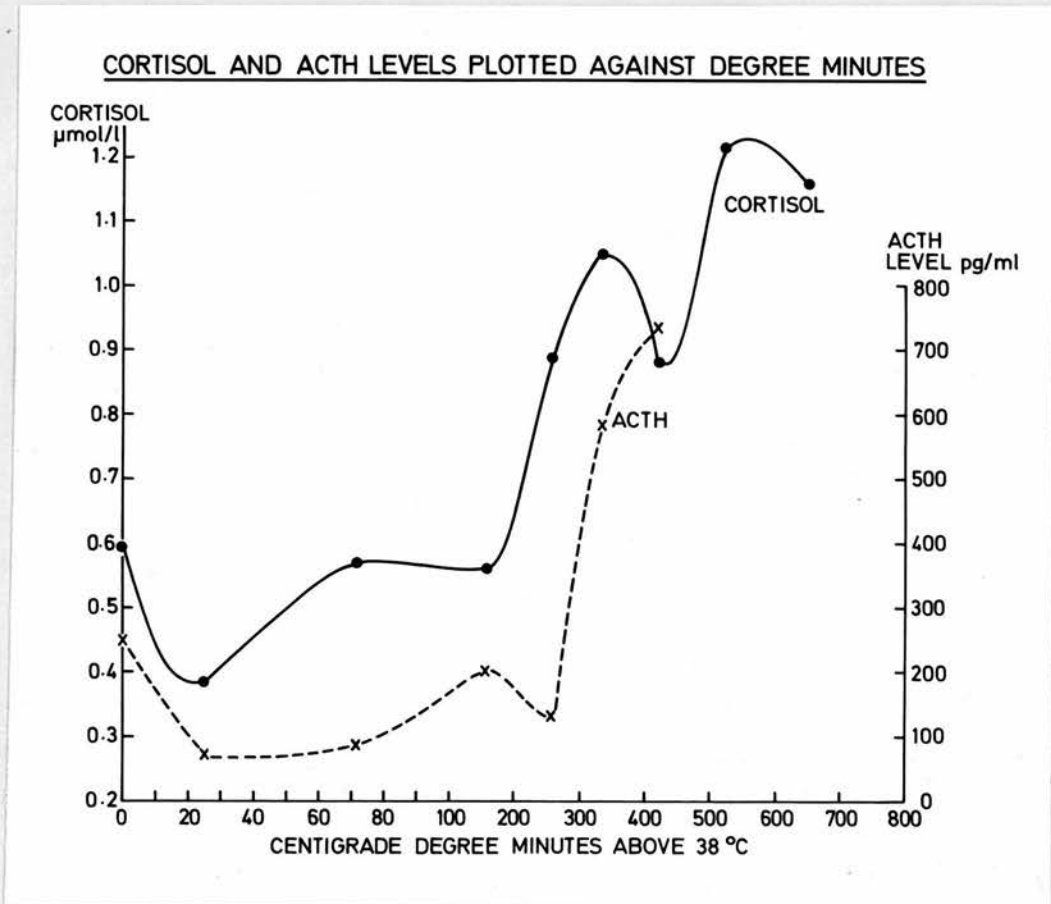


FIGURE 26

The response of plasma ACTH and cortisol was not related directly to temperature but to the quantity of thermal stress plotted here in centigrade degree minutes, 39 °C. standard.

treatment was extended beyond 4500⁰ minutes, 39⁰C. standard, the plasma cortisol did not return to the pre treatment value within 24 hours. In none of the patients was there a significant change in haemotocrit levels during treatment.

E. MARROW STUDIES AND CHANGES IN
HAEMOGLOBIN CONCENTRATION.

4.11 Sternal marrow histology before, immediately after treatment and at seven days post treatment in 14 patients showed no change with no evidence of marrow depression. The haemoglobin level fell by an average of 10% following the first treatment though the range was great (4 - 18%). Following subsequent treatments at weekly intervals the fall was less, and following each treatment there was a reticulocytosis of between 2 and 4% with a partial recovery, (Figure 27). Initial values were not attained during treatment. Osmotic fragility studies showed no evidence of change in the osmotic fragility of the red blood cell.

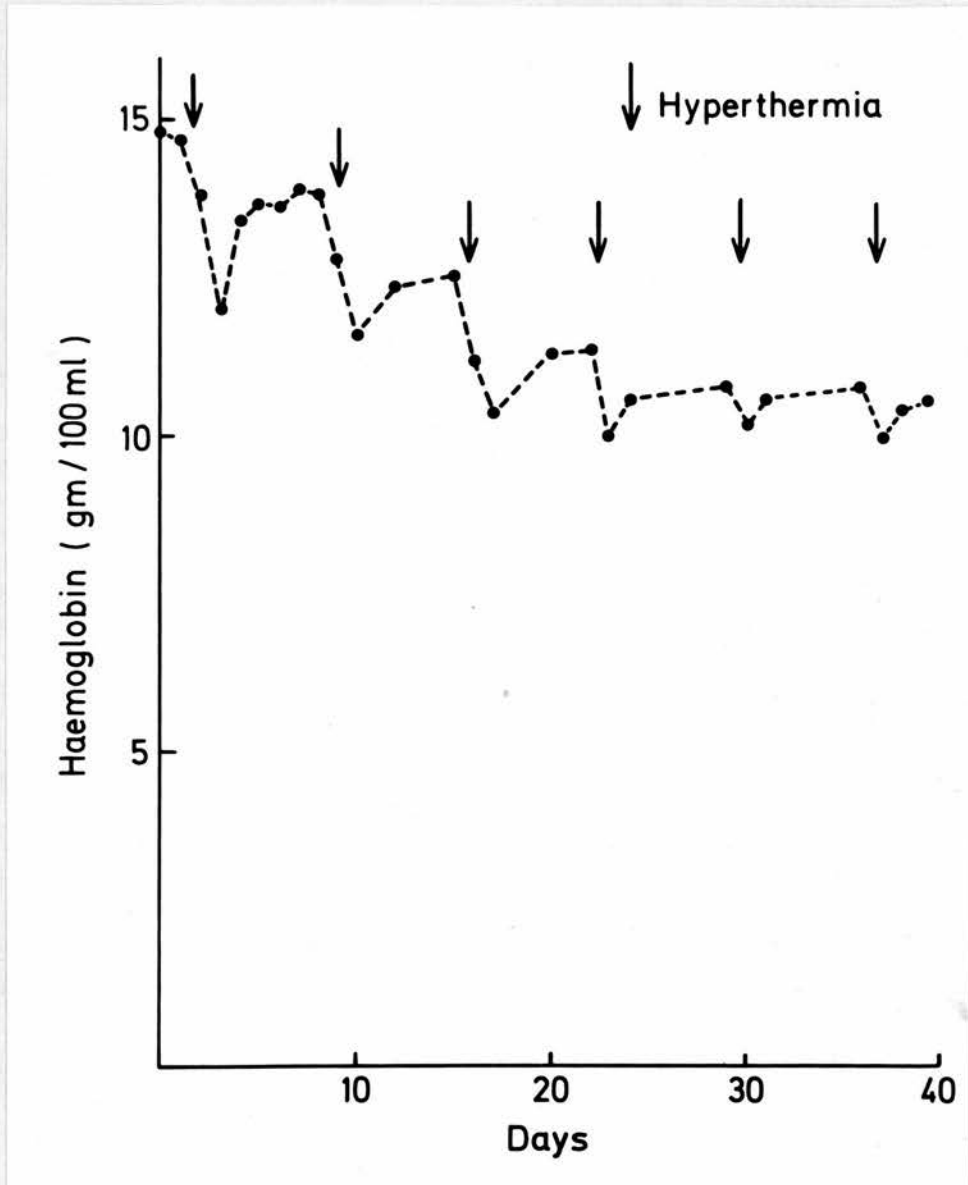


FIGURE 27.

Following each hyperthermia treatment there was a fall in haemoglobin concentration followed by a reticulocytosis and recovery. This figure is representative and is taken from one patient who was given six treatments at weekly intervals.

F. WHITE BLOOD COUNT AND THE IMMUNE
RESPONSE.

- 4.12 Figure 28 shows the average white count in 27 treatments on 21 patients. There is an initial fall in the total white count maximal at one hour followed by a rise which we treated as a maximum averaging 15.8×10^3 cells at six hours. The majority of the increase is due to immature polymorphs with no increase in the absolute lymphocyte count. The maximal response varies from patient to patient with a range of 12 - 36 x 10^3 cells at six hours. Pre treatment values were attained within seven days following treatment.
- 4.12 In all patients in whom immunoglobulin and viral studies have been observed there have been no significant changes following hyperthermia.

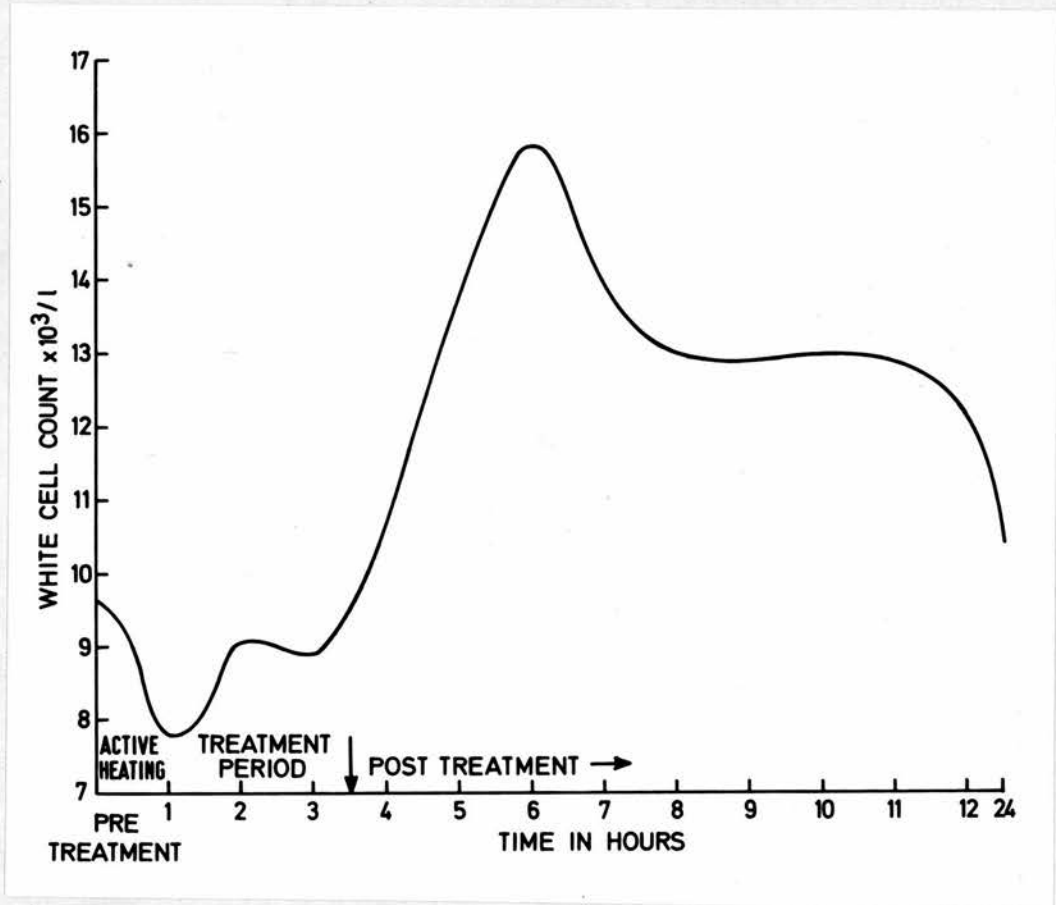


FIGURE 28.

In 27 treatments on 21 patients there was an initial fall in total white blood count followed by a rise. The figure shows the average of all these treatments though some individuals had a much higher rise post treatment.

G. INTRAVASCULAR COAGULATION; A LIMITING
FACTOR IN FRACTIONATION.

- 4.14 The most sensitive index of coagulation defect was found to be the platelet count. Other indices, such as prothrombin time, serum fibrinogen, or the level of fibrin degradation products remained stable unless there was a marked thrombocytopenia with platelet counts falling below 50,000/c.mm. following treatment. The maximal thrombocytopenia occurred at approximately 24 hours following treatment, (Figure 29.).
- 4.15 There was an initial fall in the percentage of platelets after the first treatment of less than 300 C° min., 41 $^{\circ}$ C. standard, this was less in patients with non-responsive tumours, averaging 75.7% (standard error 13.5%) as compared with those in the responsive group who averaged 45.9% (standard error 9.4%). Both groups, however, showed a degree of thrombocytopenia (Figure 30.). This was clinically reflected in the responsive group who felt unwell and were usually sick for 48 hours following treatment.
- 4.16 After an initial fraction of greater than 300 C° min., 41 $^{\circ}$ C. standard, there was an

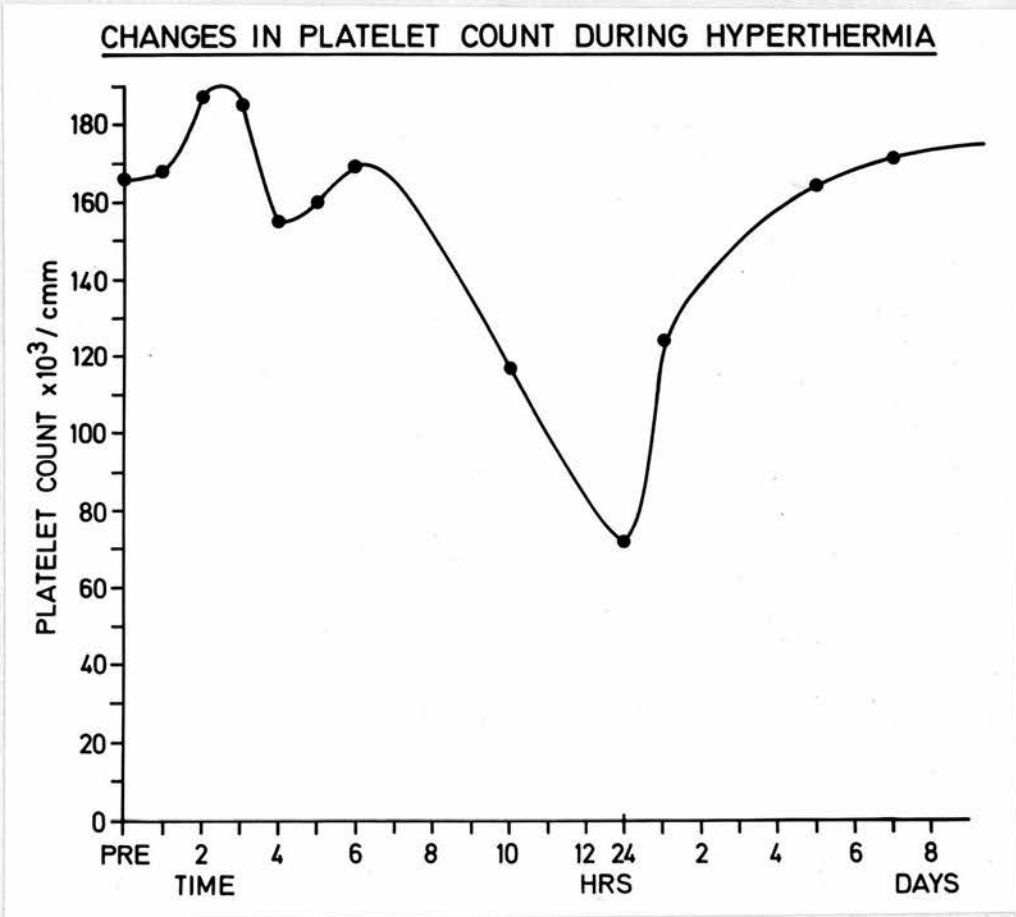


FIGURE 29.

Average platelet counts over 24 initial treatments.

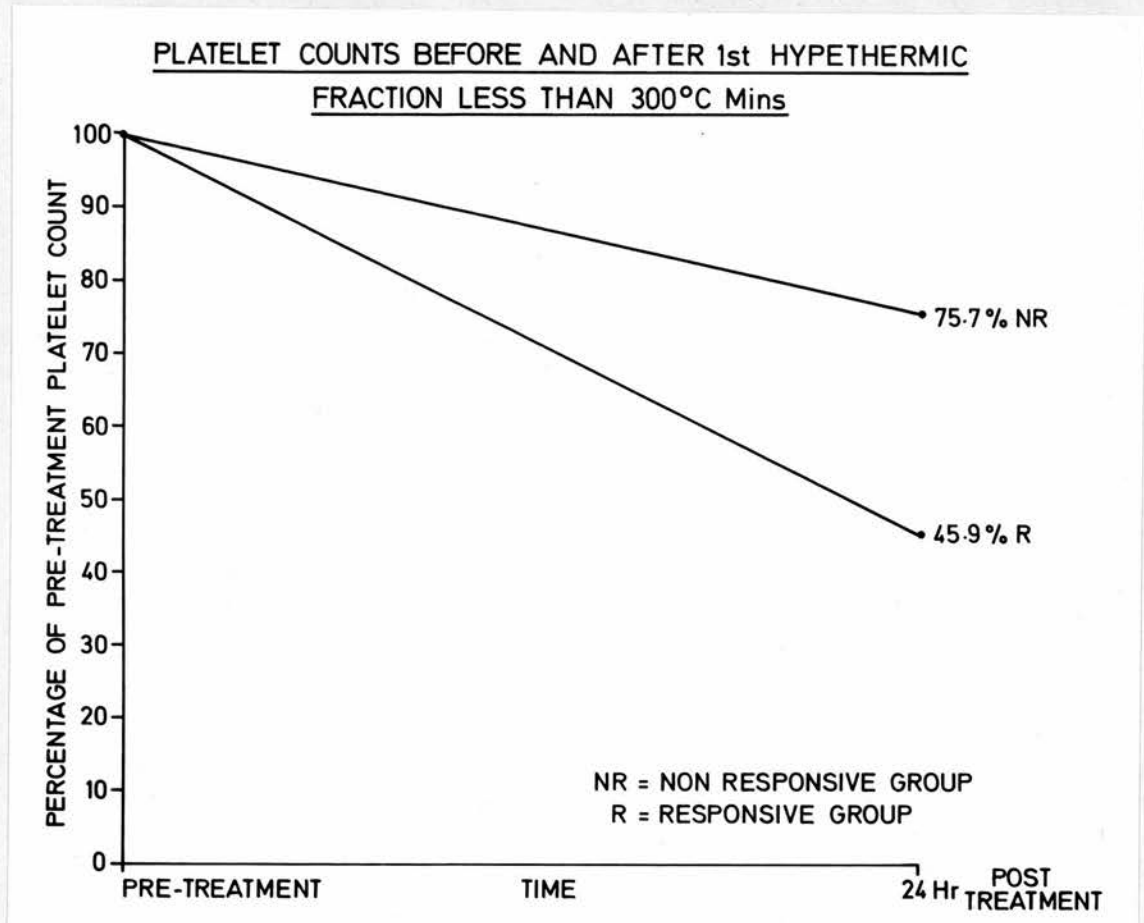


FIGURE 30.

Following an initial fraction of less than 300 Centigrade degree minutes, 41°C. standard, there was a greater fall in the platelet count of patients with sensitive tumours.

there was an increased thrombocytopenia in both the non-responsive group averaging 54.5% (standard error 17.9%), and responsive patients averaging 17.7% (standard error 11.7%) with evidence of disseminated intravascular coagulation in the most responsive tumours (Figure 31). Three such patients died with platelet counts below 10,000 and fibrinogen degradation product levels of 160 micrograms per cent, 640 micrograms per cent and 161 micrograms per cent respectively.

4.17 If a second fraction was given above 300 C.^o min. but less than 500 C.^o min., 41°C. standard, one week after the first fraction of less than 300 C.^o min., 41°C. standard, there was a rise in the platelet count in those with non-responsive tumours averaging 116% (standard error 27.7%), whereas those responding again showed a decrease following treatment averaging 58.6% (standard error 17.5%) (Figure 32.).

4.18 Two patients with non-sensitive tumours were treated at above 500 C.^o min., 41°C. standard, following a previous fraction below 300 C.^o min., 41°C. standard, there was a fall in platelets to 31,000 and 70,000/c.mm. respectively; the patient with greater thrombocytopenia required clotting factors. Neither patient returned to their pre treatment values within seven days.

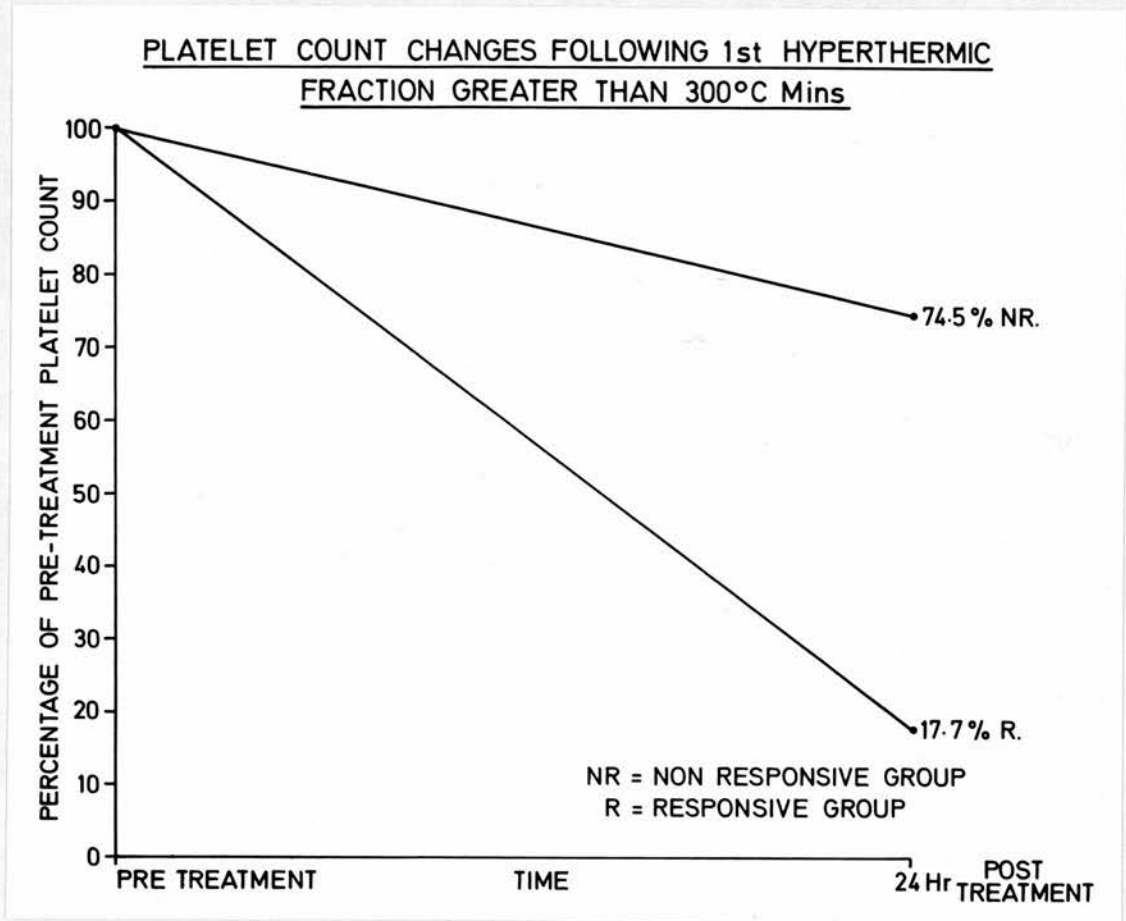


FIGURE 31.

After an initial treatment fraction of greater than 300 Centigrade degree minutes, 41°C. standard, there is a risk of inducing disseminated intra-vascular co-agulation in patients with disseminated sensitive tumours.

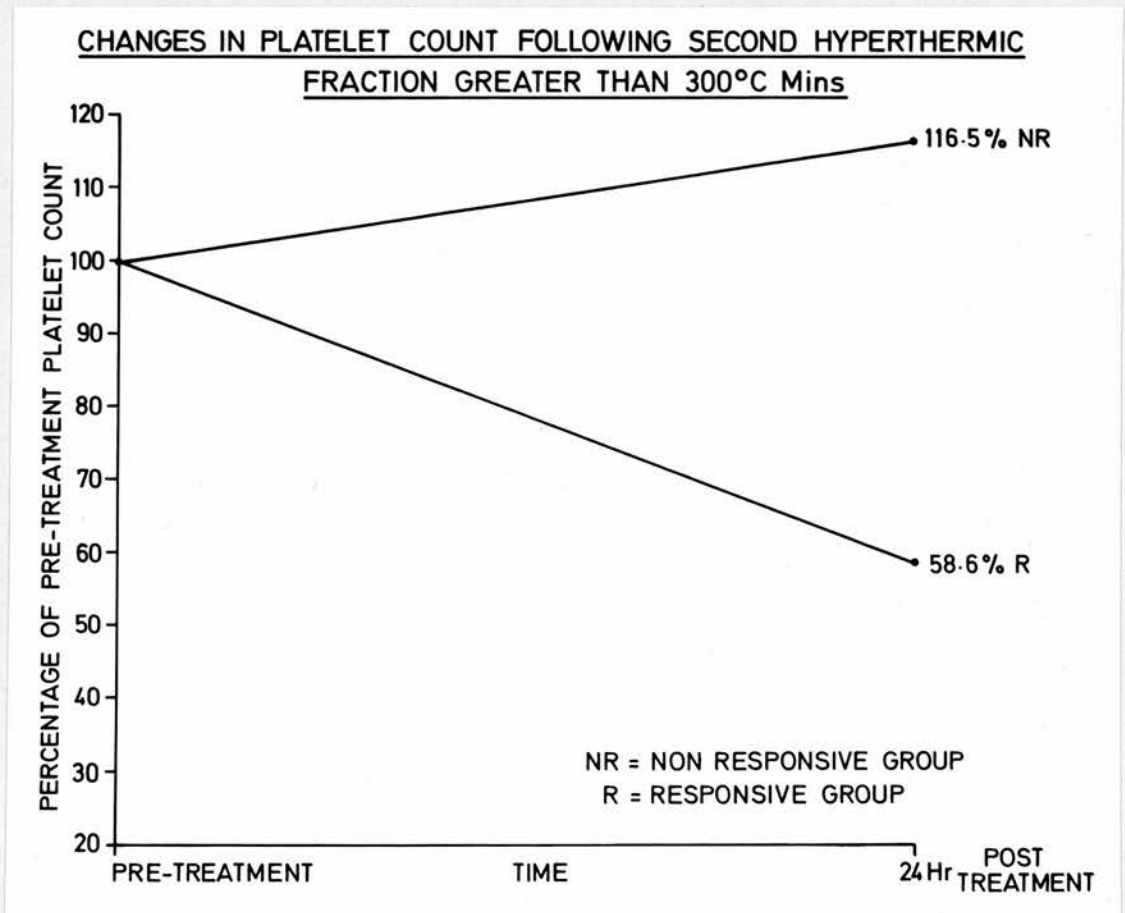


FIGURE 32.

A second fraction greater than the first given one week later resulted in a fall in platelets in those with responsive tumours, whereas those with non-responsive tumours showed a rise in the post treatment platelet count.

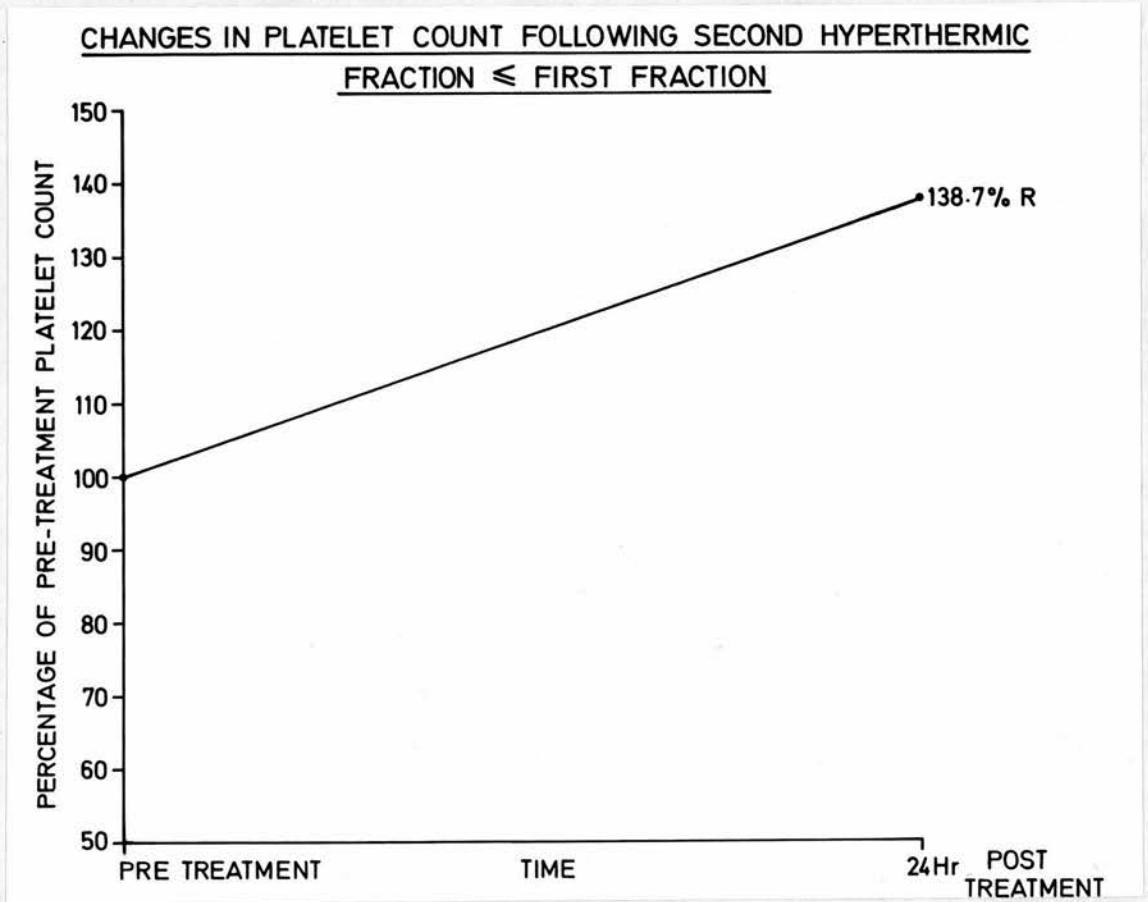


FIGURE 33.

A second fraction less than or equal to the previous fraction caused no fall in platelet count in responsive or non responsive patients.

4.19 When patients were treated with the same or a lesser fraction than previously within one week of the previous treatment there was an increase in the platelet count in the responsive group averaging 138.7% (standard error 57.6%) (Figure 33). These four patients had no nausea or sickness following this fraction. One patient with a non-responsive tumour was also treated in this manner and showed a similar rise in platelet count.

H. CLINICAL RESULTS OF WHOLE BODY HYPERTHERMIA.

4.20 62 Patients in the terminal stages of cancer, recurrent following conventional therapy have been treated by whole body hyperthermia. Each patient was considered unsuitable for further treatment by conventional means. Patients of all age groups 3 - 75 years have been treated and in 232 treatment sessions a total of 560 hours over 40°C. have been given. Patients tolerate hyperthermia well, the maximum number of treatments in any one patient being 24. 21 patients were excluded from the series, 14 were treated in developmental stages of the method when temperatures above 40°C. were not used. These patients did not respond and it is now accepted that temperatures in excess of 40°C. are

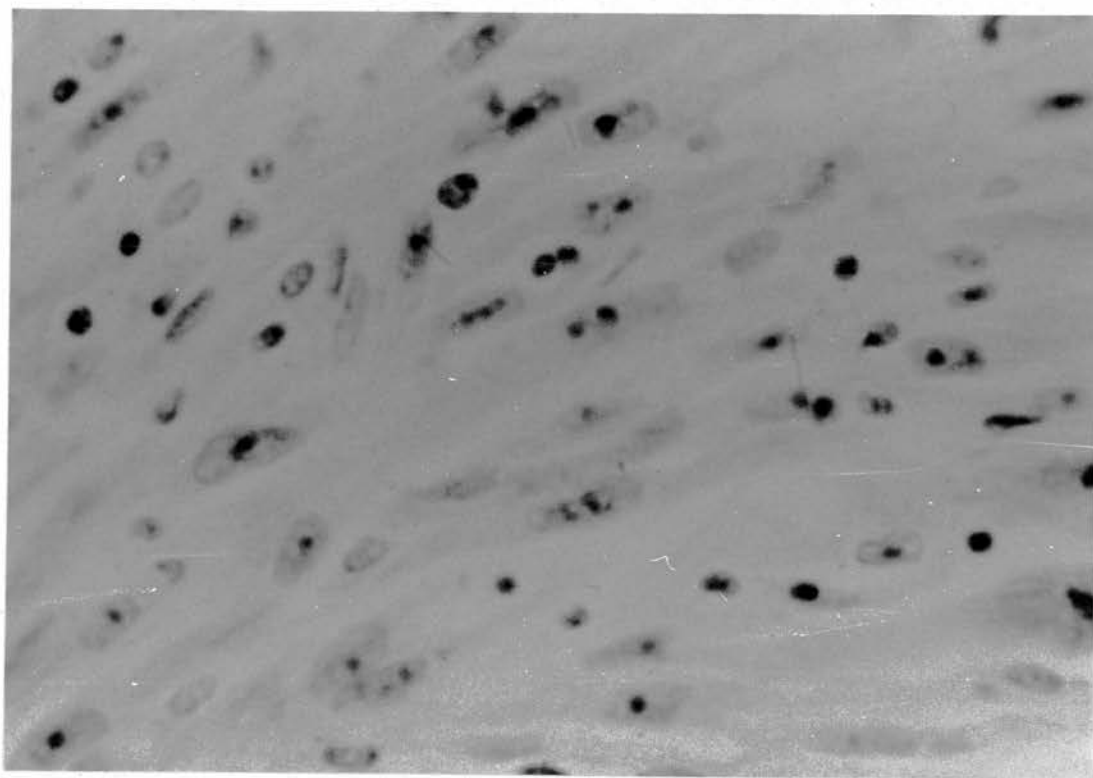
needed (Giovannella, Lohman and Heidelberger, 1970). A further 7 patients, with no obviously measurable tumour, were treated for symptomatic relief of pain alone. This series therefore comprises 41 patients. Though the numbers were small, tumours of gastro-intestinal origin and sarcomas appeared to respond more favourably than genito-urinary or breast neoplasms. Lung tumours and malignant melanomas showed an intermediate response. The cases are summarised in the following paragraphs, for a fuller description please see appendix.

Case Reports

Sarcoma

4.21 8 Cases: 6 being soft tissue sarcomas, of which 4 responded favourably to treatment. In 1 patient a lung deposit disappeared, a second showed healing of pathological fracture and a third complete regression of a fibrosarcoma recurrent in the operation scar. The fourth patient, with an advanced liposarcoma, died 24 hours after treatment. Necropsy showed recent massive necrosis throughout the tumour (Figure 34). A child with a rhabdomyosarcoma showed no response and one fibrosarcoma had subjective improvement only. Two patients with osteogenic sarcomas were treated; the first had a hindquarter amputation - two months' after the end of an 18 month course of treatment the tumour recurrent in a heat

A.



B

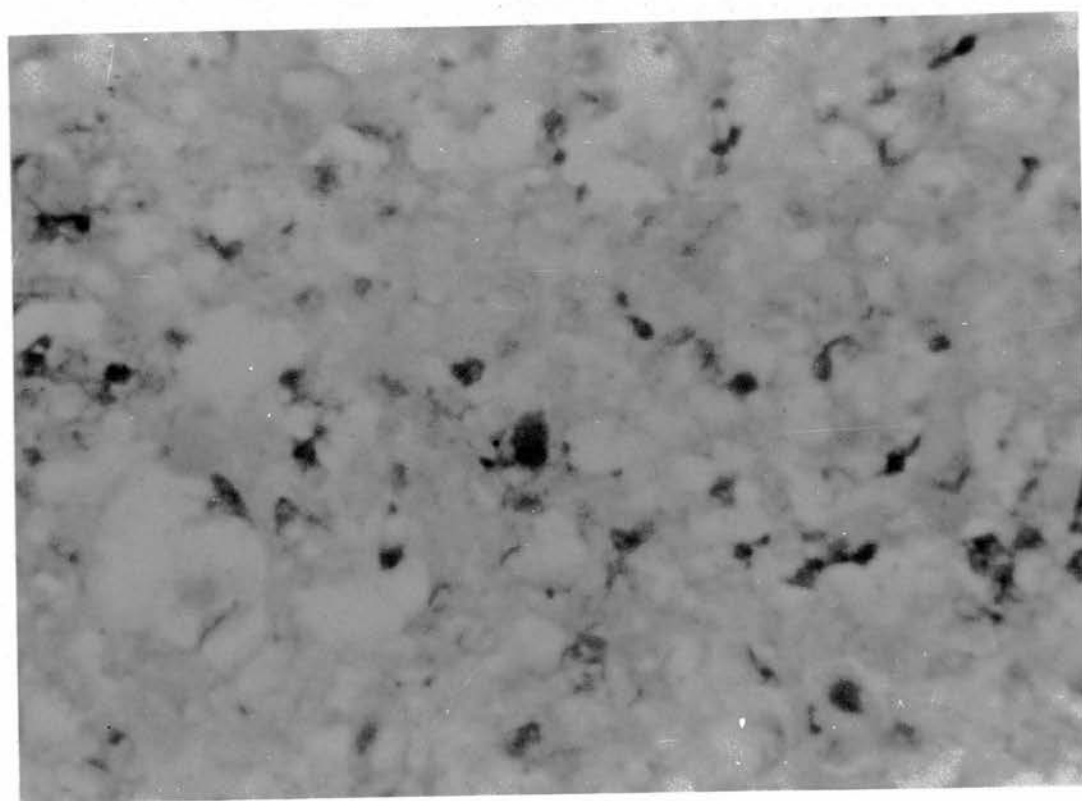


FIGURE 34

Liposarcoma A. pre treatment. B. post treatment
necropsy specimen showing gross necrosis of the tumour.

sensitive form. The second showed no response to treatment.

Carcinoma of the Stomach

4.22 4 cases: 2 anorexic patients who had been in great pain gained weight and were able to lead a relatively normal life. The third had extensive mediastinal and lung metastases and died 48 hours after treatment. At necropsy there was extensive necrosis of the tumour causing compression of the bronchus. The fourth had pain relief and weight gain but died suddenly one month after treatment with a pulmonary embolus. Necropsy was not performed.

Carcinoma of the Colon

4.23 7 cases: 1 patient had almost complete regression of massive hepatomegaly. A second had regression of skin nodules in the lower abdominal wall. One further patient had regression in tumour size. The fourth died three weeks following hyperthermia. At necropsy there was very extensive tumour necrosis. The other three showed no response.

Malignant Melanoma

- 4.24 8 Cases: In 4 there was a good initial regression of secondary deposits and pain relief. One patient had pain relief alone and three showed no response.

Carcinoma of the Lung

- 4.25 4 cases of bronchogenic carcinoma. In 1 patient with an adenocarcinoma there was regression of the primary lung tumour (confirmed at necropsy) (Figure 35), though secondary deposits remained active. The second patient, with a squamous carcinoma, showed regression of a secondary deposit in the lumbar spine. The third had relief from pain but no tumour regression. The fourth died within 24 hours of hyperthermia, prior to death there was evidence of disseminated intravascular coagulation and at necropsy there was extensive haemorrhage into the tumours.

Ovarian and Testicular Tumours

- 4.26 Two testicular teratomas and two ovarian papillary tumours showed no response to treatment.

Neuroblastoma and Nephroblastoma

- 4.27 Two children with neuroblastomas were treated. One showed a good initial response with healing of ulcerated skin over the tumour of his jaw. The second showed initial improvement till he developed a respiratory difficulty and died two days after treatment. Necropsy showed multiple haemorrhagic areas and necrosis in the tumour. One child with a nephroblastoma showed initial improvement but also died from a respiratory arrest. At necropsy there was gross necrosis of the tumour.

Breast Cancer

- 4.28 Two patients with scirrhus carcinoma were treated, one obtaining pain relief alone.

Miscellaneous Tumours

- 4.29 One case of mycosis fungoides showed initial healing and there was pain relief alone in a case of adenocarcinoma of the nasopharynx. One case each of chronic myeloid leukaemia and squamous cell carcinoma of the bladder showed no response.

A.



B.

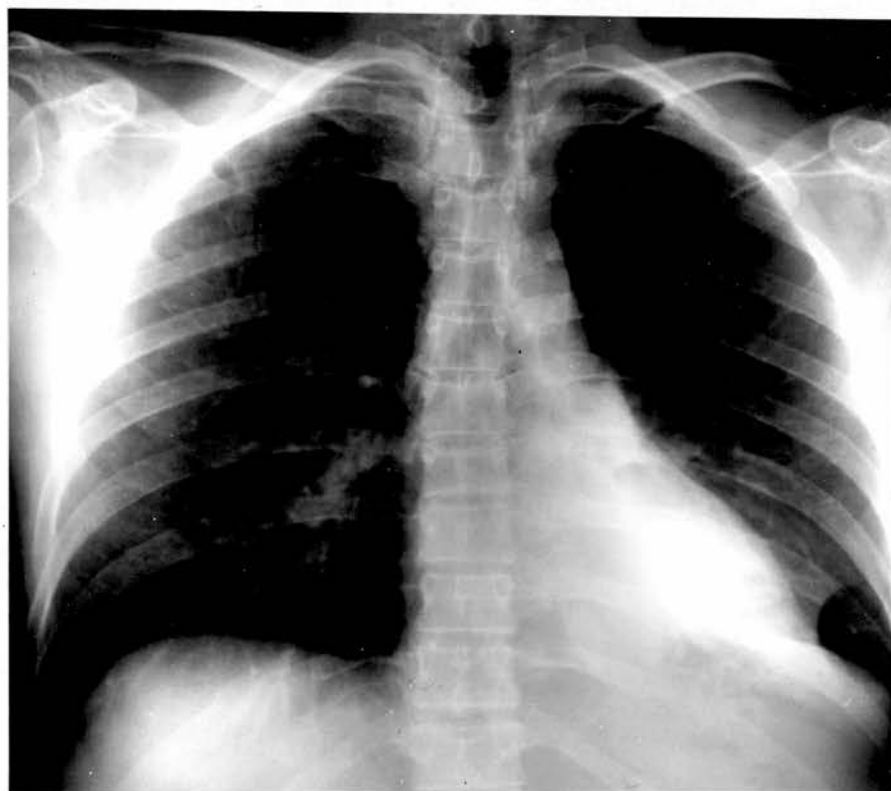


FIGURE 35.

Primary Ca. bronchus. A. pre treatment. B. following 3 increasing fractions. Though the primary resolved distant secondaries continued to grow. At necropsy some months later there was only scar tissue at the primary site.

I. COMPLICATIONS OF WHOLE BODY
HYPERTHERMIA.

- 4.30 Complications may arise from the method, from the physiological response to high temperatures or from the toxic effects of tumour breakdown and absorption. Considering that patients had been maintained in an unconscious state at temperatures over 40°C . for 560 hours, complications of major nature have been remarkably few.
- 4.31 In 1966, before the present method was in use a patient developed ventricular fibrillation and died. This was due to her temperature reaching 43°C . as a result of thermometer failure, and was the only fatality directly attributable to induced hyperthermia. The importance of several thermometers has been stressed; these should be placed at different levels in the rectum and oesophagus and read out on at least two separate systems. The highest reading thermometer is taken as the true reading.
- 4.32 50% of the patients developed circumoral herpes simplex following the first treatment but not after subsequent treatments (Figure 36). Sore throats, pressure sores due to prolonged immobilisation during treatments and superficial burns in oedematous, hypoproteinaemic patients



FIGURE 36.

50% of patients developed circumoral Herpes simplex after the first treatment but not after subsequent treatments.

may be minimised by adequate padding and air mattresses. For grossly oedematous patients the wax temperature was decreased to 45°C.

- 4.33 Five adult patients died within 48 hours of hyperthermia. Their deaths were associated with evidence of disseminated intravascular coagulation. In 3 necropsy showed recent tumour necrosis. This is a reported complication of aggressive chemotherapy in the presence of widespread sensitive tumour and indicates the necessity of fractionating hyperthermia in the presence of disseminated sensitive tumour.
- 4.34 Two children with advanced neoplastic disease died shortly after treatment. They were given opiates for the relief of distress and died of respiratory complications. In both post mortem show gross tumour necrosis and respiratory difficulties are a reported complication of disseminated intravascular coagulation (Hardaway, 1973). Another patient died from fibrosing alveolitis, possibly due to repeated exposure to the hot, moist ventilating gases then in use (Henderson, 1971), or as a result of treatment with bleomycin, 6 months previously. Hot, dry gas is not used and this complication has not recurred. No patient has developed uric acid nephropathy due to increased

cellular breakdown but patients developing raised uric acid levels were routinely placed on Allopurinol.

SECTION 5.DISCUSSIONA. MECHANISM OF ACTION OF HEAT STRESS

- FEVER THERAPY
- CELLULAR RESPIRATION
- NUCLEIC ACID SYNTHESIS
- PROTEIN SYNTHESIS
- CELL CYCLE EFFECTS
- MEMBRANE EFFECTS
- REPAIR

B. FACTORS EFFECTING HEAT SENSITIVITYC. DOSAGE AND THE UNITS OF HYPERTHERMIC STRESSD. ADVANTAGES OF THE METHODE. RESULTS

- HETEROGENETY OF TEMPERAURE
- ANAESTHESIA DURING HYPERTHERMIA
- HAEMODYNAMIC RESPONSE
- PLASMA ELECTROLYTE AND ENZYME LEVELS
- LIVER FUNCTION, A LIMITING FACTOR IN MAXIMAL THERAPEUTIC TEMPERATURE
- PLASMA CORTISOL LEVELS: THE STRESS RESPONSE
- HAEMATOLOGICAL CHANGES
- INTRAVASCULAR COAGULATION, A LIMITING FACTOR IN FRACTIONATION.
- IMMUNE RESPONSE AND VIRAL STUDIES.

F. CLINICAL RESULTS OF WHOLE BODY HYPERTHERMIA

A. MECHANISM OF ACTION OF HEAT STRESSFever Therapy

5.1. The induction of fever by means of infection or repeated injection of purified toxins has resulted in some complete regressions of tumours, (Nauts, 1953). The method of action may be divided into the effects of hyperthermia, stimulation of the immune response, and, possibly, a direct toxic effect on the tumour cells by bacterial endotoxins and exotoxins. It is likely that those patients who had the greatest pyrexia and the highest regression rate also reacted more vigorously to the exogenous antigen and were immunologically more competent. It is not possible to separate these factors but cancer treatment by each of the three methods has its advocates.

5.2 The effects of immunotherapy with B.C.G. or *Corynebacterium parvum* are poorly understood, and the role of chemotherapy is constantly changing. By understanding the mechanism of action of each limb it may be possible to combine different modes of treatment so that maximal potentiation of tumour cell death recurs with the least possible damage to the normal cell.

Cellular Respiration

5.3 Inhibition of oxygen uptake occurs in

sensitive tumours at temperatures over 40°C . (Dickson and Shah, 1972). Precht (1973) suggested that this was due to a reversible denaturation process which after prolonged incubation becomes irreversible. Bowler (1973) found a good correlation between the lethal dose for heat stress and the loss of respiratory control with a decrease in oxidative phosphorylation in the mitochondria. There are considerable variations in the heat sensitivity of the oxydative phosphorylation in the mitochondria from different organs. Brain mitochondria being the most heat resistant.

- 5.4 Christiansen and Kvamme (1969) found that electron transport in the succinate cytochrome 'C' region of the mitochondria is inhibited by hyperthermia together with a loss of respiratory control and uncoupling of phosphorylation. They also demonstrated leakage of endogenous cytochrome 'C' during hyperthermia from the mitochondria. Overgaard, (1975) showed that mouse mammary carcinoma cells have a pronounced decrease in cytochrome 'C' oxidase activity after incubation at 42.5°C . for one hour. Mondovi (1969) working with Novikhoff hepatoma cells was able to show that the addition of cytochrome 'C' enhanced oxygen consumption at 42°C . and reduced the inhibitory effects of heat. Total disruption of the cells abolished the differential heat sensitivity of normal and tumour cells.

- 5.5. The differential heat sensitivity of aerobic respiration in different cell types is therefore dependant on the integrity of the mitochondria and the cytochromes maintained in ordinary sequence on their cristae. Anaerobic respiration is decreased but not completely inhibited by heat (Muckle and Dickson, 1971). During hyperthermia there is diminished aerobic glycolysis and increased reliance on anaerobic glycolysis with a subsequent decrease in intracellular pH. (Overgaard, 1974). There is therefore a decrease in production of adenosine triphosphate to power the intracellular function such as synthesis and transport across the cellular membranes.

Nucleic Acid Synthesis

- 5.6. The effects on supranormal temperature on nucleic acid synthesis was first studied in viruses. Lwoff (1962) established a critical thermosensitive event involving hydrogen bonds in the viral cycle and advanced the idea that the event was the polymerisation of a monomer polymerase protein. A viral structural gene carried the information for the synthesis of this monomer which can be synthesised at supranormal temperatures but cannot be polymerised into an active polymerase.

- 5.7. Gharpure (1965) showed that fifteen minutes at 45°C. would inhibit the growth of DNA viruses but not RNA viruses. The heat sensitive step appeared to be DNA dependant RNA synthesis necessary for the transcription of DNA virus specific protein. This would agree with Simard and Bernhard's (1967) findings who suggested that the decreased rate of DNA synthesis was due to heat damage of sensitive hydrogen bonds responsible for the structural integrity of the DNA double helix, this damage blocking transcription. Depression of RNA synthesis at 42°C. may add to the inhibition of DNA synthesis as RNA is necessary for the synthesis of late replicating DNA and for the mitotic process to take place (Warocquier, 1969).
- 5.8. It is not possible at present time to affect to polymerase enzyme and inhibit DNA transcription in this way. Alkylating agents by reacting with the sulphdryl groups of DNA, replacing the hydrogen ion with an alkyl radical, block transcription in a similar way to that suggested for hyperthermia. It is likely that such drugs would cause an additive effect with hyperthermia.
- 5.9. Resistance to alkylating agents is in part due an increased capacity for repair of damage to the DNA (Crawthorn and Roberts, 1968). Inhibition of repair enzymes may result in the continued sensitivity of a tumour to alkylating agents and thus has possible clinical significance in

tumours which are initially very sensitive to such agents.

5.10. RNA synthesis by transcription of DNA, takes place both in the nuclear cytoplasm forming messenger RNA, and in the nucleolus with the production of ribosomal transfer RNA; Nucleolar changes have been studied in depth. The sensitivity of the nucleolus to heat is reflected by a loss of granular ribonuclear protein and nucleolar structure including the vacuoles. Similar structural changes have been found to occur with a number of antimetabolites (Love, Studzinski, et al, 1965). Transcription of the nucleolar RNA from the organiser chromosome results in the synthesis of 45s molecules which are the precursors of ribosomal RNA. This transcription is blocked by actinomycin D which causes a loss of granular and fibrillar nucleoprotein, in the nucleolus (Stevens, 1964).

5.11. Warocquier and Scherrer, (1969) studied the sedimentation analysis of labelled nucleoproteins after incubation at 42°C. and were able to show a build up of pre ribosomal RNA. This is seen in the cell as an overaccumulation of fibrillar ribonucleoprotein. No evidence of granular ribonucleoprotein formation was found suggesting a completely different mode of action to Actinomycin D. Following heat stress, ribosomal RNA was selectively inhibited with

defective conversion of the 45s preribosomal RNA to the functional 28s and 18s species. The primary lesion appeared to affect the processing of preribosomal RNA to functional ribosomal RNA which fails to appear in the cytoplasm.

- 5.12 This might be due to formation of altered preribosomal RNA as a consequence of faulty primary, secondary, or tertiary structure, or to the thermo sensitivity of the enzymes responsible for the conversion. Non ribosomal RNA appears in the cytoplasm at the normal rate and there is only a slight decrease in synthesis of messenger RNA in Hela cells at 42°C. At higher temperatures cells show an overall 50% decrease in the RNA production with an arrest of messenger RNA transfer to the cytoplasm (Heine, Sverak et al).

- 5.13 Simard and Bernhard (1967) found that the nucleolar loss of granular ribonuclear protein was reversible and was probably due to uncoiling of the ribonuclear protein to form a fibrillar variety. Feldherr (1973) suggested that irreversibility in the later stages may be due to leakage of ribonuclear protein from the nuclear envelope with the development of cytoplasmic inclusion bodies, shown by Love et al in 1970 to consist of aggregations of ribosome like granules. High levels of ribonuclear protein in the nucleus during heat stress has a protective effect (McCormick and Penman, 1969).

- 5.14 Chemotherapeutic drugs which act by inhibiting RNA synthesis, therefore act in a different manner to hyperthermia. Purine antagonists, such as 6 mercapto purine, or more specifically 5 fluorouracil, might be expected to potentiate the effect of hyperthermia. This effect has been shown to occur in Hela cells (Palzer and Heilelberger, 1973).

Protein Synthesis

- 5.15. Protein synthesis is inhibited at temperatures above 40°C. in heat sensitive cells (Mondovi, Agro et al, 1969). The disaggregation of polysomes with resultant decrease in protein synthesis which occurs may prevent synthesis of repair enzymes required for replacement of enzymes naturally degraded. This process may be of benefit in combating viral infections of the cell by denying the virus amino acids necessary for viral synthesis (McCormack and Penman, 1969).
- 5.16 The importance of inhibition of protein synthesis as a cause of cellular death following heat stress is unclear. Palzer and Heidelberg (1973), were able to show that the inhibition of protein synthesis by cyclohexamide prior to hyperthermia exerted a protective effect on the

cells. If inhibition of protein synthesis is protective, it would explain the resistant phase shown by cells subjected to a second episode of hyperthermic stress within a few hours of the first.

- 5.17 Gerweck and Dewey (1975), have explained this resistant phase in terms of cell cycle effects. They suggested that cells progressed to a more resistant phase of the cell cycle. A third alternative is that as hyperthermia recruits cells from the G_0 phase into cycle (Kal, 1975, Dickson and Calderwood, 1976), there may be fewer cells left capable of entering into the cycle.

Cell Cycle Effects

- 5.18 The most sensitive phase of the cell cycle to hyperthermic killing is during DNA synthesis of the 'S' phase. During this period the number of chromosomal aberrations induced by hyperthermia is similar to an equivalently lethal dose of X-rays (Dewey, Westra, et al, 1971). Cells heated in either mitosis or G_1 had an aberration frequency lower than that observed following a correspondingly lethal dose of X irradiation. This would suggest that cells die in 'S' phase primarily because of an effect on DNA production

resulting in chromosomal aberrations.

5.19 In mitosis and G_1 this was not true as very few chromosomal abnormalities were induced and these were far too low to account for the fraction killed. The primary effect on the sensitive mitotic cell probably involves heat denaturation of spindle protein (Sisken et al, 1965). This conclusion is based on the observation that cells heated in metaphase are unable to complete mitosis and appear in the next division in the tetraploid state (Dewey et al, 1971). The damage during G_1 results in less cell killing than in 'S' phase because chromosomal aberrations induced during 'S' phase tended to result in the loss of large pieces of genetic material (Westra and Dewey, 1971). This fragmentation of the DNA has been studied mainly in bacteria and viruses.

5.20 Bacterial and viral DNA when heated in vitro sustains both single and double strand breakages (Bridges, Ashwood-Smith and Munson, 1969). The effect on the nucleic acid is by disruption of the secondary structure, held together by non-covalent bonds, such as hydrogen bonds, and cleavage of the N - glycosidic end of the phosphate-ester bonds.

5.21 Breakage of the non-covalent bonds leads to a very abrupt change in the conformation in the double helix and dissociation of the individual

complementary strands. Within a narrow range of temperature this reaction is rapidly reversible (Szybalski, 1967). Cleavage of phosphate-ester bonds causes single strand breaks which are normally repairable.

5.22. A further effect which has been shown to occur during slight temperature increases in the cell in hydrolysis of N - glycosidic bonds of purine deoxy nucleotides in DNA. This causes a loss of biological activity as a sequel to depurination (Eigner, Boedtker and Michaels, 1961). Thermal inactivation of RNA is also in part caused by cleavage of phosphate-ester bonds (Szybalski, 1967).

5.23. Damage to covalent bonds causing strand breaks can normally be repaired in the cell by using the complementary DNA strand as a template and replace the missing or modified bases. If not repaired the free radical ends may react with surrounding molecules making repair impossible.

5.24 Alvarez(1973), has shown that tumour DNA is more sensitive to heat damage than normal DNA. This may explain the difference in heat sensitivity of some cancer cells. Deletions in the X chromosome, which is the site of a nucleolar organiser are very frequent, and may be responsible for subsequent changes in nucleolus.

Membrane Effects

5.25 Cellular membranes are formed of a lipoprotein complex and their precise integrity and structure has to be maintained for the function of their attached enzymes. In the crayfish, Bowler, Duncan et al, (1973) were able to show a correlation between the temperature for death of the whole animal, the impairment of membrane bound Mg^{++} ATPase, and the control of membrane permeability. Oxydative phosphoryllation in mitochondria is uncoupled at temperatures between $41 - 45^{\circ}C.$, and the cytochrome C. content of the medium increases due to damage of the mitochondrial membrane and subsequent leakage. Christiansen and Kvamme (1969), found that ascites tumour cells were more sensitive than liver cells in this respect.

5.26 Emmelot and Bos (1968), studied the temperature sensitivity of both intact cells and isolated membrane enzymes from normal rat liver and from hepatomas. They found that the rat hepatoma cells were more heat sensitive than the normal rat liver cells. There were clear differences between the liver and the hepatoma cells in the specific activities and the ATPase enzyme systems. They concluded that as the $Na^{+} - K^{+} - Mg^{++} - ATPase$ systems are lipoprotein complexes forming part of the cell membrane, the differences between liver and hepatoma sensitivity

were due to differences in chemical and morphological composition of the membranes rather than to changes in the proteinaceous enzymes themselves. Changes in either the lipid or the enzymes attached has been suggested by Miura, Kawashima et al, (1971) as the reason for hyperthermic fragility of human leukaemic cells. The heat energy of inactivation of such proteins is within the capacity of hyperthermic therapy (Westra and Dewey, 1971).

5.27 Of clinical interest is the observation that some chemotherapeutic drugs are able to enter the sensitive cells more easily at higher temperatures due to these membrane changes (Hahn, 1975).

5.28 Damage to lysosomal membranes could cause leakage of toxic enzymes and products into the cytoplasm, however this does not appear to be a primary method of cell killing by hyperthermia (Turano, Ferrard, et al, 1970). There is a decrease in intracellular pH which would activate lysosomal enzymes once released. This together with ease of diffusion of toxic products through the damaged membrane might account for the rapid absorption of toxic material which occurs during hyperthermic therapy.

Repair

5.29. Inhibition of repair of sublethal damage may be due to heat destruction of the enzymes concerned, a block in their synthesis, their inactivation by alteration in structure, or by

lack of energy supply in the form of ATP required for reactions. Damage to DNA during hyperthermia has been shown to be due in part to loss of repair of single strand breaks caused by heat or alkylating agents. Rejoining of such induced breaks in the DNA is inhibited above a critical temperature which is about 41.5°C . for human skin cells (Bronk, Wilkins and Regan, 1973).

- 5.30 Hochacka (1969), and Somero (1969), have provided evidence that acclimatisation to a new temperature involves changes in the kinetic properties of enzymes, probably by the production of different isoenzymes. Different thermal sensitivities for cells of varying origin can be explained on a differing thermal sensitivities of contained enzymes.

B. FACTORS AFFECTING HEAT SENSITIVITY

- 5.31. In 1934 Crabtree and Cramer noted that "most neoplasms are badly vascularised and, if they are large, unequally vascularised". Different parts of a growth are therefore supplied with oxygen in a varying degree so that they will vary in their susceptibility to a given dose of radiation". This statement is also true of hyperthermic therapy as nutritional status, contact inhibition, and the number of

cells in active cycle governs their sensitivity. Like other forms of tumour therapy, cells can become resistant to heat therapy.

- 5.32. Studies at a cellular level have shown that in heat resistant cell lines there is a decrease cellular permeability, reflected by decreased leakage of materials such as uridine following heat stress, combined with an accelerated rate of repair of heat induced damage (Reeves, 1971). A change in membrane permeability would agree with the lipoid theory and fit with the fact that the inhibition of thymidine uptake by DNA is abolished in cell free preparations (Modovi, Agro et al, 1969). Increased repair rates due to a build up of reserves of RNA as suggested by McCormick and Penman, (1969), or the production of a specific RNA, would promote cellular function under conditions of heat stress.
- 5.33. There appears to be two mechanisms of resistance to heat stress, the first, acting after acute heat shock in which the cells are heat tolerant for 17 - 25 hours, and probably represents cells arrested in a heat tolerant phase of the cycle (Gerweck and Dewey, 1975), but may represent protection endowed by inhibition of protein synthesis as discussed earlier. The second is reflected by an increased heat resistance in daughter cells, and may be genetically controlled though there are no obvious chromosomal changes (Harris, 1967 b).

C. DOSAGE AND THE UNITS OF HYPERTHERMIC STRESS.

5.34 The treatment dosage given at any one time has the objectives of causing maximal tumour damage with the least possible damage to normal tissue. Each fraction should be large enough to prevent the induction of resistant clones of tumour cells. The heat stress required to kill a fixed percentage of cells, usually taken as 67% (D_{37} value), has been shown by Westra and Dewey, (1971) to be directly related to temperature and time above a critical temperature.

5.35 The critical temperature represents that temperature above which cells are irreversibly damaged and co-incides with the beginning of the straight line portion of the survival curve. Each system has its own characteristic survival curve as shown by Adolf (1947) for the whole animal, and by Orr (1955) for different tissues. Each survival curve has its characteristic critical temperature.

5.36 To state treatment dosage in terms of temperature and duration of treatment is therefore insufficient to standardise the degree of heat stress to which the system has been subjected.

In most cell systems, at temperatures above the critical value, for each 1°C . rise in

temperature the time taken to produce the same degree of thermal damage is halved. If treatment is standardised to the time taken at 1°C . above the critical temperature to produce the same degree of thermal stress as the actual treatment a clinically useful unit of heat stress can be defined. The centigrade degree minute is the time equivalent at 1°C . above the critical temperature for a specified cell system under specified conditions required to produce the same degree of thermal stress in that system.

- 5.37 Unfortunately the heat sensitivity of human tumours has not been charted though some preliminary studies have been carried out by Dickson and Suzanger, 1976. The usage of the degree minute would enable comparisons of tumour and normal tissue sensitivities to be made. A tumour which requires $800\text{ }^{\circ}\text{C}$ minutes at 41°C . standard would also be cured by 400 minutes at 42°C . or 200 minutes at 43°C . If the normal surrounding tissue requires $1000\text{ }^{\circ}\text{C}$ minutes at 41°C . standard for cell death, there is a reasonable differential of heat sensitivity. If the effect of hyperthermia is potentiated $\times 2$ by a given dose of radiotherapy or a standardised drug concentration then the centigrade degree minutes required for cell death can be halved. In this

way the hyperthermic fraction can be easily calculated and any potentiating factors allowed for. In the clinical situation the tumour temperature is difficult to keep constant and there are changes when the tissue is heating up and cooling down. The dosage in degree minutes can be calculated in terms of a single value in spite of changing temperature, and has the advantage of stating the critical temperature of the system described.

5.38 In this thesis the degree minute 41°C . standard was used for tumour therapy by whole body hyperthermia and assumes a critical value for all tumours treated of 40°C . This is certainly inaccurate, some tumours may have a lower critical value, some, such as ovarian and testicular tumours must have a critical value considerably above this level. 40°C . was assumed as this was the temperature at which inhibition of oxygen consumption began in sensitive tumours in vitro (Dickson and Shah, 1972).

5.39 Whole body hyperthermia produced stress as reflected by an increase in ACTH and cortisol levels at temperatures above a critical value of 38°C . Heat stress to the whole body was therefore standardised in centigrade degree minutes 39°C . standard, and assumes a linear relationship for heat stress between temperature and time above 38°C .

D. ADVANTAGES OF THE METHOD

- 5.40 The advantages of using molten wax as a source of heat are considerable. It is cheap and easy to obtain, and as it does not come into contact with the patient, is re-usable. Wax is an insulator and there is no danger of short circuiting electro-radiograph electrodes or conducting should the need for defibrillation arise.
- 5.41 Wax adjacent to the patient solidifies forming an insulating barrier from the rest of the molten wax at 50°C . There is a large thermal reservoir of molten wax but a skin temperature of approximately 44°C . is achieved, allowing a maximal permissible thermal gradient. As the molten wax cools it gives out latent heat of solidification so tending to keep the average temperature of the liquid wax constant. This method therefore gives the maximum possible rate of increase in body core temperature.
- 5.42 Once the required increase in body temperature is achieved the molten wax can be syphoned off leaving an insulating pellicle to conserve heat loss. The absence of water vapour allows this pellicle to be opened to a varying degree allowing the evaporation of sweat with resultant cooling of the patient. In this

way the body core temperature can be maintained to within 0.1°C . The only disadvantage of using wax is that it is difficult to clean up spills.

5.43 The method is safe and only one patient has died during hyperthermia. This was the second patient treated in 1966, when only two thermometers were used, and the patient's core temperature reached 43°C . due to thermometer failure. With 8 thermometers at different sites registering onto 2 different recording systems the possibility of thermometer failure is very remote.

5.44 Patient care is easy using this method as the patient is easily seen and can be easily exposed for clinical examination. If at any time it is required to stop treatment the patient can easily and quickly be lifted out of the bath and left wholly exposed, resulting in a rapid fall in body core temperature.

E. RESULTS

Heterogeneity of Temperature

- 5.45 Patients are closely monitored during treatment and the temperature of the body core taken as the highest reading of the eight thermometers used. During active heating the temperature in the oesophagus is 1 - 2 centigrade degrees higher than in the rectum (Figure 13). At stable temperature the oesophageal and rectal temperatures are the same and agree with the value for the tympanic recording. The elevation of oesophageal temperature during active heating probably reflects the local heating effect produced by ventilating with heated gases.
- 5.46 During other forms of pyrexia in man variations of temperature in different parts of the body have been observed. The intra-cardiac temperature may be as much as 0.8°C . below the rectal temperature (Eichna, Berger, et al, 1951). The temperature of blood draining from the liver and brain indicate that these organs are at a higher temperature than the intra-cardiac reading, but the exact temperature difference is not known (Aschoff and Wever, 1958). Some preliminary work by Budinger (1976) in rats suggests that in this species the liver may be as much as $1 - 2^{\circ}\text{C}$. above the measured rectal temperature. In the

present series the rectal, tympanic and oesophageal temperatures were the same at stable temperature and would suggest some average homogeneity of temperature, but precise organ differences are not known.

Anaesthesia During Hyperthermia

- 5.47. At temperatures over 40°C . patients become restless and anxious (Wallace, 1943). Increasing muscular activity makes external thermoregulation more difficult and uses the patient's reserves of energy and increases the rate of lactic acid production. Patients were therefore paralysed and ventilated during treatment, light narcosis being maintained by intermittent opiates and short acting barbiturates.
- 5.48. At temperatures over 40°C . there is a rapid increase in the quantity of anaesthetic agents required to maintain mild narcosis, and at the end of the treatment session there was a large quantity of drugs circulating resulting in prolonged anaesthesia. The use of a high epidural anaesthetic decreases the need for systemic narcotics, and by causing peripheral vasodilation, increases the rate of heat absorption from the skin.
- 5.49 One patient following epidural anaesthesia

with intermittent marcaine developed motor weakness in both legs. Marcaine is stated by the makers to be stable at the temperatures involved, however, it has not been used subsequently. 2% plain Xylocaine was routinely used for the epidural anaesthetic and there have been no complications responding from its use. McKenzie (1975) using the same method of inducing whole body hyperthermia has used low dose Halothane and reported no side effects.

Haemodynamic Response

5.50 Heat death in humans is usually caused by failure of the heart and blood vessels (Cloudsley-Thompson, 1963). In uncontrolled hyperpyrexia both hypoglycaemia and dehydration have adverse effects on the heart and circulation. Dehydration results in an increased workload on the cardiovascular system. The blood volume is reduced and the viscosity of the blood increased. In addition the peripheral blood pool space expands in response to attempts by the body to move core heat to the skin. Eventually, the flow is reduced to the point that removal of heat and waste products from the tissue and delivery of energy substrates to the tissue is so impaired that death ensues. Inducing controlled whole

body hyperthermia necessitates careful monitoring with accurate control of body core temperature as well as fluid and electrolyte balance to ensure maintenance of an adequate cardiac output and tissue perfusion.

Changes in Heart Rate

- 5.51 Damato, Lau et al (1968) found that in unacclimatised conscious volunteers heat stress caused a decrease in arterial and central venous pressures due to peripheral vasodilation resulting in compensatory increase in heart rate. During hyperthermia the increase in heart rate was only 60% of that found in conscious patients (Tanner, 1951). This can be explained by anaesthetic depression of the sympathetic cardiac stimulatory fibres as a reduction of the depth of narcosis leads to a further rapid increase in the heart rate. That the mechanism is neurological, as suggested by Cooper and Kerslake, (1955), is supported our finding of a decrease in eventual rate at stable temperature under epidural anaesthesia. The increase in heart rate per centigrade degree rise in temperature is less during active heating when the body core temperature is rapidly raised, this implies a limit to the rate at which the compensatory mechanisms can respond to changing temperature. With epidural blockade

the increase in heart rate was less suggesting that sympathetic drive may be more important than vagal release.

Cardiovascular Response

- 5.52 At body core temperatures above 40°C . there is a hyperdynamic circulation which is dealt with by different species in different ways, and may account in part for the differences in thermal tolerance between species. Hales and Dampney (1975) showed that in dogs there is a marked increase in cardiac output with a large degree of arterio-venous shunting of blood, whereas sheep, which are more resistant to thermal stress, reacted largely by redistribution of blood flow to central organs. There is an increase in cardiac output of 30% at 40°C . in man (Demarto et al, 1968), which is due to an increase in pulse rate as there is a fall in stroke volume (Rowell and Murray, 1969).
- 5.53 In terms of hyperthermic stress the blood flow to any particular organ is important, but also the temperature of that organ, which may be different to the core temperature, the degree of arterio-venous shunting in the organ concerned, and its metabolic rate, determine its heat sensitivity. The pattern of blood flow distribution in man during hyperthermia is not well known

but some studies have been recorded. At normal temperatures a decreased arterial blood carbon dioxide tension (Pa CO_2) has been associated with a decreased cerebral blood flow (Reivach, 1969). Colton and Frankel, (1972) showed that in dogs this was also true during hyperthermia, but because of the decrease in overall vascular resistance, cerebral blood flow increased during hyperthermia. Snodgrass and Lorenzo (1972), found between a 6 - 11% increase in cerebral blood flow per degree rise in temperature up to 41°C . Nemoto (1970), found that cerebral hypoxia did not develop in hyperventilated dogs at 42°C . In man treated for venereal disease by whole body hyperthermia to 41°C . cyanosis was a constant feature with marked mental changes (Wallace and Gushby, 1945).

- 5.54 In 1968 Rowell, Brengelman et al, showed that in heat-stressed man at 40.2°C . there was a reduction in hepatic and splanchnic blood flow with an increase in the hepatic lactate production indicative of anoxia. In 1970 working with a group of young university students who were heated to temperatures in the right atrium of 39°C ., Rowell et al found a decrease in total peripheral resistance but an increase in splanchnic resistance. Hepatic and renal blood flow was again decreased in spite of an increase in cardiac output. In view of these findings our method was

modified in that the patients were hyperventilated with oxygen enriched air, thus ensuring that any blood going to a hypoxic region such as the liver would carry as much oxygen as possible. By ventilation the muscular work of breathing is reduced and it was hoped to reduce lactic production in this way. Plasma is infused at a body core temperature of 41.8°C . to compensate for the increased blood pool volume, 40 mgs. of Lasix is given towards the end of treatment session to ensure that there was no circulatory overload as venomotor tone is regained during cooling.

Plasma Electrolyte and Enzyme Levels

5.55 The lethal effects of hyperthermia by inhibition of cellular metabolism together with increased membrane permeability involved changes which are intracellular. In dogs, Spurr and Barlow, 1970 measured tissue electrolytes and found that after heating to a rectal temperature of 42.5°C . for one hour there were significant increases in the concentration of sodium in the liver, jejunum, and brain, potassium in the heart and skeletal muscle, and also an increased water content in the jejunum. Some changes are reflected by changes in the extra-cellular fluid and show as changes in plasma electrolytes and

enzymes, whose concentrations which can be measured. In the present series it was hoped that by maintaining the fluid and electrolyte balance and plasma electrolyte concentrations within the physiological range, that the normal cell would have the best possible conditions to withstand heat stress. In particular it was hoped that this would avoid myocardial irregularities.

Fluid and Electrolyte Losses

5.56 Without replacement, electrolyte changes occur in hyperthermic animals (Spurr and Barlow, 1959), and in Dr. Pettigrew's earlier treatments there were significant changes in these levels. Fluid losses during hyperthermia were great, averaging over 550 mls/hour of which 90% is lost in sweat (Figure 37). However, this sweat rate is similar to that reported in unacclimatised subjects exercising in a hot climatic chamber, and although the sweat sodium concentration of 84 m.mol/l is higher than the reference range of 20 - 70 m.mol/l, similar increased sweat sodium concentrations have been reported during prolonged heavy sweating in unacclimatised subjects (Furman and Beer, 1963). It was possible to achieve a satisfactory overall salt and water balance by monitoring the serum electrolyte concentrations during hyperthermia therapy. Using

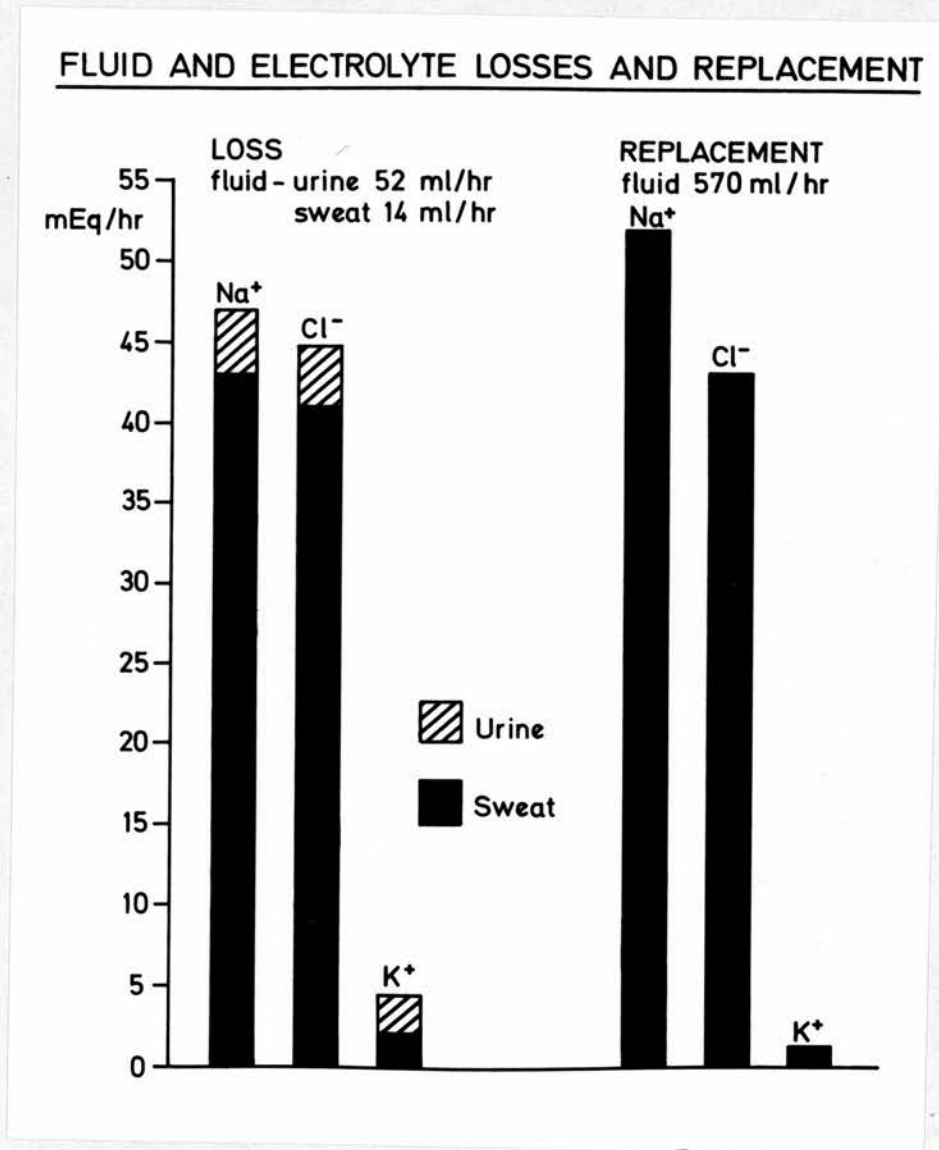


FIGURE 37.

Fluid and electrolyte losses are great during hyperthermia but are adequately replaced by the infusion regime now used.

a standard replacement regime running at 500 mls. per half hour and giving Lasix if necessary to maintain a urinary output of over 100 mls. per hour, the serum electrolytes are now maintained within normal limits.

Liver Function, A Limiting Factor in
Maximal Therapeutic Temperature.

5.57 Following fever therapy for neurosyphilis or gonorrhoea, jaundice was a frequent occurrence, usually appearing after the second day and being of brief duration. MacDonald (1944) reported an incidence of 19% and transient hyperbilirubinanaemia was detected in 75% of King, Wallace and Nicol's (1943) series, and in all of the cases studied by Wallace (1943). A detailed account of the post mortem changes in 17 fatal cases was made by Gore and Isaacson, (1949) who found that in the early post hyperthermic period there was liver congestion with cloudy swelling and glycogen depletion of parenchymal cells. This was followed by nuclear and cytoplasmic vacuolation and the accumulation of fat droplets. Those patients who died more than 48 hours after hyperthermia had all developed clinical jaundice and there was centralobular necrosis of the liver which became increasingly severe as the survival period lengthened.

- 5.58 It appeared that the liver was the most heat sensitive organ in the human body. These findings could be due to the increased temperature of the liver over the body core temperature (Budinger, 1976), or due to anoxia resulting from the decrease in hepatic blood flow. In order to investigate this and to establish the maximum safe therapeutic temperature in man a series of liver function tests and enzyme studies were carried out.
- 5.59 In animals Burger (1970) has shown that there is a critical core temperature of 42°C . above which there is an increase in serum enzyme activity. In Adolf's (1947) series studies the rate of death of mammals became time related at body core temperatures over 42°C . The serum enzyme activities in man in our series also increases at temperatures over 42°C ., and, although isoenzyme studies were not carried out to prove that the hepatic origin of these enzymes, the relationship to liver function in the form of decreased excretion of B.S.P. is suggestive of liver origin. Perfusion of the isolated cat's liver by Rawlinson and Kellaway, (1944), indicated a critical level of 41°C . for hepatic thermal injury, and it is therefore probable that this is a direct thermal effect.
- 5.60 We were not able to protect against the development of enzyme changes by the use of glucagon or with hyperbaric oxygen at two

atmosphere pressure. These changes in serum enzymes were taken to indicate a maximum safe therapeutic temperature of 41.8°C . giving a 0.2°C . margin of safety. This was subsequently adopted for all later treatments.

Plasma Cortisol Levels: Stress Response

5.61 Collins in 1969 subjected 12 fit young male volunteers to environmental heating inducing a gradual rise of oral temperature to 39°C . over a period of two hours. During this there was a rapid fall in the plasma cortisol maximal at one hour followed by a sharp rise. If the fall in plasma cortisol was temperature related, it might be prolonged by the higher temperatures used in hyperthermia therapy.

5.62 The second factor which prompted this study was the possibility of either adrenal malfunction or the occurrence of adrenal metastases in patients with disseminated cancer. It was undertaken to evaluate adrenal function during hyperthermic therapy and to see whether the routine replacement hydrocortisone therapy previously given was necessary during treatment. Serial plasma cortisol levels were estimated in eight patients during fifteen sessions, and serum adrenocorticotrophic hormone (ACTH), in four of these patients during five treatment sessions.

- 5.63 The observed changes in plasma, ACTH and subsequent cortisol levels, of an initial fall at temperatures above 38°C . followed by a sharp rise occurred in all patients. This includes one patient treated on three occasions in whom no epidural anaesthetic was technically possible.
- 5.64 Pre-medication and muscle relaxants have been shown to have no significant effect on plasma cortisol levels during surgery (Hammond and Vandam, et al, 1958). This was also the case in our series in which two of the patients had no premedication and showed the same cortisol response as those premedicated. Opiates have been shown to block the release of ACTH at the hypothalamic level (Vandam, and Moore, 1960). However, as opiates were given on several occasions during treatment without apparent effect, it is probable that this effect is not significant during stress.
- 5.65 Figure 25 showed the plasma ACTH and cortisol levels against temperature showing that the lowest point for cortisol at about $39.5 - 40^{\circ}\text{C}$. The graph is very similar to that of Collins, Few et al, (1969), who subjected volunteers to a lower rise in body core temperature (39°C) and found a similar fall in plasma cortisol at above 38°C . which lasted for one hour, followed by a rise. The rate of disappearance of ACTH would be

in keeping with inhibition of synthesis, and decay of that already in circulation, as the half life of ACTH is eight minutes (Gallagher, Yoshida, et al, 1973). Similarly, the subsequent fall in plasma cortisol is also accounted for by decay as it has a half life of 70 minutes (Gallagher et al, 1973). The rise in ACTH to pretreatment values takes nearly 45 minutes to occur and would appear not to be entirely temperature dependent.

- 5.66 This conclusion is supported by Collins et al (1969) previous studies, in which the time taken for the cortisol level to return to pretreatment value was one hour. This would suggest that the cortisol response is dependant on hyperthermic stress rather than an absolute temperature level. The posterior hypothalamus mediates both heat retention and the discharge of ACTH from the anterior pituitary (Hensel, 1973). There would appear to be an inter-relationship between thermoregulation and adreno-cortisol function mediated by ACTH release, inhibition being followed by a resetting event after a period of time. Increasing fractions of treatment above 450 centigrade degree minutes, 39°C. standard, causing a further resetting event with higher levels of ACTH and cortisol. This would suggest that with increasing fractions,

even with adequate fluid and electrolyte replacement, there is a increase in the total body stress and that there must be a maximal treatment in terms of temperature and time that can be tolerated.

- 5.67 Though these results are crude in that ideally to measure ACTH levels samples should be taken within the half life of ACTH in order not to miss a secretory episode, and few patients were monitored, the trend after an initial fall is for a rise in plasma cortisol levels to supra-normal levels. There is therefore no need during hyperthermic therapy for the replacement steroids, but as there is a stress response induced by this form of treatment, adrenal function requires to be checked prior to treatment.

Haematological Changes

- 5.68 No treatment of cancer is wholly selective. The toxic effects of the treatment limit the maximum permissible dose. Figure 38 is an extension of the Johnson's work (1940), and compares the heat sensitivity of tumours and normal tissue from in vivo and in vitro studies. The thermal death times for each tissue lies within a band of temperature and time. It is probable that at 42°C. approximately 20 hours of treatment would be required for total tumour destruction of a sensitive tumour. It is not known what dose in terms

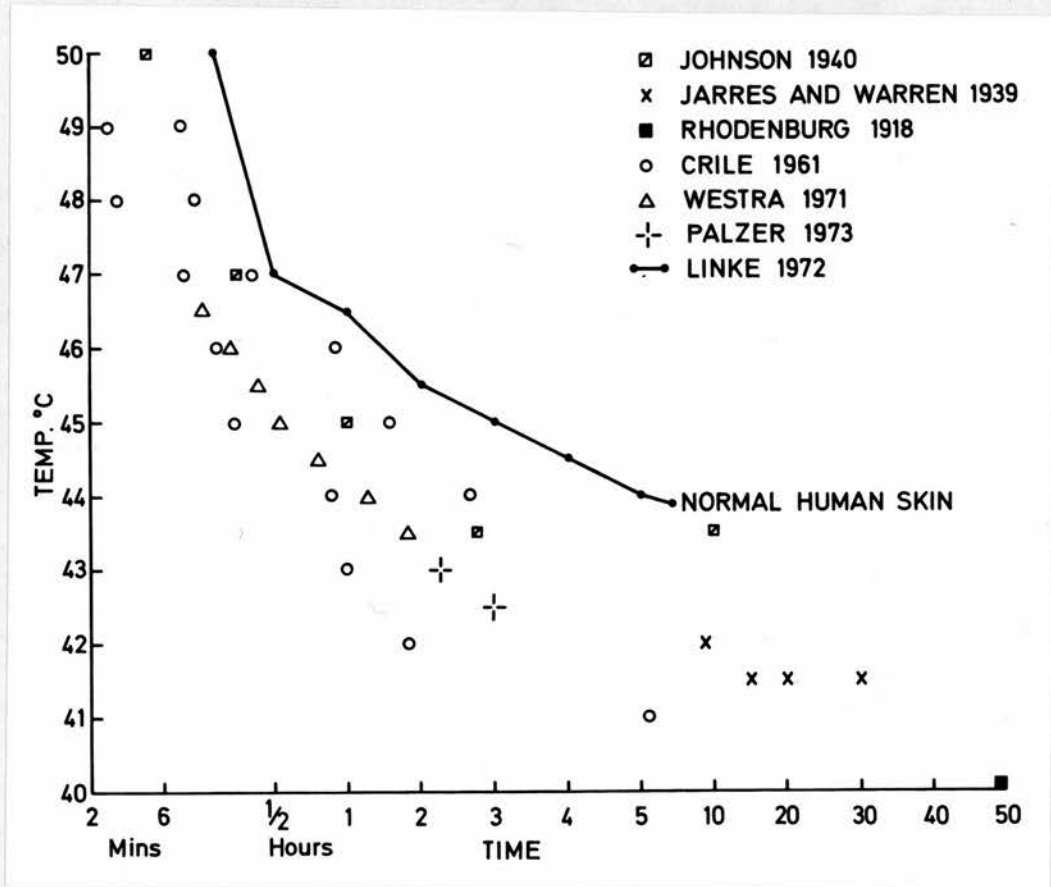


FIGURE 38.

Temperature/Time for 100% necrosis of various tumours in animals as compared with that for normal human skin.

of time at 41.8°C . could be tolerated in cancer patients or what the limiting factor in hyperthermia would be. Cohen and Warren, (1935) treated patients for up to 20 hours at 41.5°C . for venereal disease. Measurements of oxygen consumption during treatment in our unit proved too insensitive to limit treatment on this parameter, and a more sensitive index was required. For this reason the blood forming elements and blood coagulation was studied in depth.

Marrow Studies and Changes in Haemoglobin

- 5.69 In cancer therapy the side effects are caused by toxicity to normal tissue. Cells in cycle are affected by all groups of drugs and by X-rays, and one of the most frequently affected regenerating cell systems in the body is the bone marrow. As hyperthermia acts mainly on cells in cycle, the effects on the bone marrow might indicate the degree of toxicity of hyperthermia.
- 5.70 The marrow histology, from biopsies taken before and after hyperthermia, together with the reticulocytosis which developed between treatment fractions, indicate an active marrow with no depression. Another regenerating system in the body is gut epithelium. We were not able to study this although some patients did develop a

form of diarrhoea following prolonged hyperthermia. The results indicate that either the marrow is not sensitive to the hyperthermia, or more likely, that the degree of hyperthermic stress induced was insufficient to produce a toxic effect.

- 5.71 There was a 10% fall in the haemoglobin following each treatment, which was probably due to the destruction of ageing red cells as demonstrated following hyperthermic treatment in rabbits (Karle, 1969). If the damaged ageing red cells are rapidly removed by the reticulo-endothelial system during treatment it might account for the unexpected absence of an increase in osmotic fragility of the red blood corpuscles during treatment.

White Blood Count

- 5.72 Most causes of fever are associated with a rise in peripheral white blood count and this has always been considered as part of the body's defence to infection. Most cancer therapy causes leucopenia, the white count was therefore studied to assess the relative importance of these two opposing parameters, and to see whether there was any significant fall in the white blood count following hyperthermia which might necessitate antibacterial cover.

- 5.73 The dramatic increase observed in the white cell count during treatment remains unexplained. Leucocytosis following artificial fever was reported in 1935 by Cohen and Warren, following treatment of venereal disease, and the pattern of this response was the same as our own. Immature polymorphonuclear leucocytes cause the rise in overall white count, but the origin and fate of these leucocytes is not known. The leucocytosis is not maintained following treatment and subsequent leucocytosis may be indicative of infection and should be investigated as such. At the present time Mr. A. P. Gee is studying the immunocompetance of these leucocytes, the proportion of lymphocytes, and whether they are capable of attacking tumour cells.

Intravascular Coagulation, A Limiting Factor
in Fractionation.

- 5.74 Increased vascular coagulation has been a reported complication of malignant hyperpyrexia (Purkis, Horrolt et al, 1967), and is a known complication of aggressive chemotherapy in the presence of wide spread sensitive tumours (Levy, Benham-Kahn, et al, 1970). Peck and Reiquam (1973). found that cancer patients have a delicately balanced coagulation mechanism which might easily

be tipped towards a low grade chronic disseminated intravascular coagulation by any changes in this balance.

5.75 Three adult patients died within 24 hours of hyperthermia with evidence of coagulation defect, with low platelets and raised fibrin-fibrinogen degradation product levels. For this reason the coagulation status of the subsequent patients treated was studied and related to the amount of treatment they had received. The degree minute, 41°C . standard, was taken as the unit of therapeutic hyperthermia and was defined as the time in minutes multiplied by the temperature in degrees centigrade that the body core is at a temperature above 40°C ., and represents an equivalent time in minutes spent at 41°C .

5.76 After a first hyperthermic fraction of less than 300°C min . there was a fall in platelets in both responsive and non-responsive patients. The non-responsive group had no further decrease in platelets when treated by a second, larger fraction. This would indicate some initial destruction of normal tissue which may be due, in part, to the destruction of ageing red cells, as shown by Karle (1969). Subsequent greater fractions can be tolerated following a first smaller fraction. In the patients with non-responsive tumours treated above 500°C min ., 41°C . standard, there was a marked thrombocytopenia which may represent too

large an increment in fraction dosage ($200\text{ C}^{\circ}\text{ min}$, 41°C . standard), or a maximum to the degree of thermal stress tolerable by the whole body.

5.77 The shape of the survival curve of cells during hyperthermia has been shown to be similar to that following X-ray irradiation, having a shoulder indicating repairable damage before an exponential phase is reached (Westra and Dewey, 1971). The action of hyperthermia in destroying cells is largely by damage to enzyme systems necessary for the repair of otherwise sub-lethal damage (Hahn, 1974). Cells are either killed or return to their previous state following each treatment. Each treatment at weekly intervals, was not additive and a second, larger fraction was required to produce the same fall in platelet count. This may be due to the remaining population being more resistant to further therapy. Clinical results reflected the platelet count, in that patients having a second fraction, similar to the first do not have a large fall in platelet count and feel well immediately following the second treatment.

5.78 Cytotoxic chemotherapy and hyperthermia have been shown to potentiate each other (Hahn, 1975). The only death due to D.I.C. in a patient treated with an initial fraction of less than $300\text{ C}^{\circ}\text{ min}$., 41°C . standard, occurred following chemotherapy

given at the same time as hyperthermia. In subsequent cases this combination has been used after two fractions giving the chemotherapy along with the third hyperthermia fraction, of the same duration as the second. Three patients were treated with closely monitored low dose heparin to try and avoid platelet consumption. Patients with sensitive tumours however, bled into the tumours during treatment, and this form of preventative therapy was therefore discontinued. In two patients with over 500 C⁰ min, 41⁰C. standard, resulting D.I.C. has been successfully treated by an infusion of platelets and clotting factors.

5.79 The present result would agree with previous work that the most obvious effect of acute D.I.C. in patients with cancer may be thrombocytopenia alone. This may be explained by an increased production of consumed factors in cancer patients (Peck and Reiquam, 1973). During hyperthermia the patients are in a hyperdynamic vasodilated state and there is a consequent rapid absorption of any toxic breakdown products resulting from cellular death. There is some breakdown of normal, probably ageing tissue during hyperthermia, and if the load of toxic products is increased by a breakdown of sensitive tumour then disseminated intravascular coagulation may be precipitated.

5.80 The present results have been on too few patients

to be significant and also represent a random selection of tumour types, which may have differing sensitivities to hyperthermia. The trend however, is towards a consumption coagulopathy in patients with sensitive tumours. There is therefore a need for fractionation in hyperthermic therapy but the frequency of treatment and the exact heat fractions to be used in each individual tumour are still to be assessed.

- 5.81 No patient, treated without cytotoxic chemotherapy, with a first fraction of less than $300\text{ C.}^{\circ}\text{ min.}$, 41°C. standard, has developed a serious degree of thrombocytopenia (Figure 39). This fraction was therefore taken as the maximum for a first fraction, and there have been no deaths following hyperthermia using this fractionated type of therapy. Treatment is planned in degree minutes and a circle of thread, of known area is pinned to the temperature chart during therapy. Using this technique the planned number of degree minutes can be given.

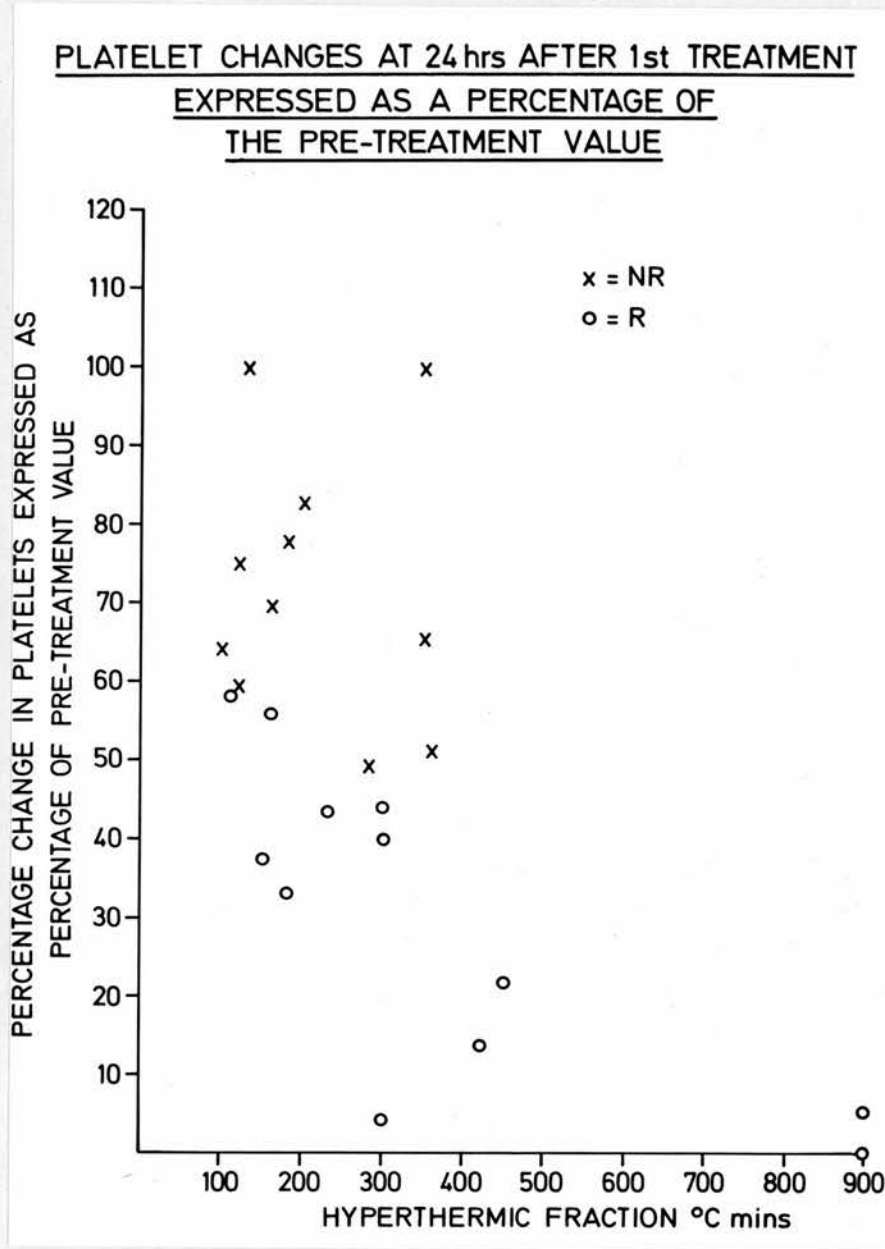


FIGURE 39.

No patient treated with a first fraction of less than 300 C.° min., 41° C. standard, developed a serious degree of thrombocytopaenia. The one patient with low platelets at 300 C.° min., 41° C. standard was also given chemotherapy and was included for completeness.

Immune Response and Viral Studies

5.82 In 1972 one patient, with disseminated melanoma, developed a marked lymphadenopathy with associated pyrexia ten days following hyperthermic therapy. This continued for five days following which the pyrexia settled and the lymphadenopathy completely regressed as did the tumour. Further it was noticed that 50% of patients developed Circumoral Herpes simplex following the first treatment but not following subsequent treatments (Figure 36). The possibility that latent virus was being activated or extruded from the cells during hyperthermic stress, or that hyperthermia might be stimulating an immune response to the tumour was considered. As there is a possibility of a viral aetiology to some tumours (Culliton, 1972), and Mondovi, Santoro et al (1972), had shown an increase in the immunogenicity of heat treated cells, all subsequent patients were investigated for changes in immunoglobulins and by viral antibody titres.

5.83 The absence of an immune response in these patients may reflect the large bulk of tumour present and the poor clinical state of the patient. There is a considerable difference of opinion regarding the effect of hyperthermia on the immune response. Crile (1963) found that tumours

heated in foot pads of mice could be cured. However, if the same tumour was minced up immediately after heat exposure and injected into another animal it grew almost as though it had not been heated. Goldenberg and Lancer, (1971), found that local heating of colonic human tumours implanted into one cheek pouch of a hamster inhibited growth of a similar tumour in the contro-lateral pouch, suggesting some stimulation of the immune response. Dickson (1976) on the other hand suggested that even local hyperthermia may decrease the immune response and prepare the ground for metastatic spread. However he was not able to apply local heat without inducing whole body hyperthermia and there was a 90% death rate following local treatment. He was unable to cure tumours over 3 cms. in diameter by heat alone. The activity of the immune response following hyperthermia requires further evaluation and no definite results have as yet been published. These studies are being carried out on Dr. Pettigrew's patients by Mr. A. P. Gee and form the basis of his Ph.D. thesis.

F. CLINICAL RESULTS OF WHOLE BODY
 HYPERTHERMIA.

- 5.85 In 1965 Dr. Pettigrew started to induce whole body hyperthermia using a water bath. In his initial series few patients were treated at above 40°C., and these cases were therefore excluded from the review. Those patients treated before 1972 were assessed on reports in the case notes. This was not satisfactory as all the patients were terminally ill with disseminated disease and had no further therapy planned. Notes were often scanty and measurement of size of tumours and degree of regression following hyperthermia rarely noted. Where no comment of improvement was made it was assumed that there was no response and the patient placed in the non responsive group.
- 5.86 From 1972 to the end of the study, the review sheet was completed at regular intervals, however, it was still difficult to evaluate a moderate response, or lack of progression of disease. These groups were again classed as non responsive. This method of documentation was carried out in the hope of isolating one or two tumour types which might be very sensitive to hyperthermic therapy and to which this labour intensive form of therapy might offer cure.

5.87 There were no cures following hyperthermia alone, poorly differentiated tumours such as fibrosarcoma showed evidence of massive tumour necrosis following therapy. Similarly two children with neuroblastoma and one with a nephroblastoma showed massive tumour necrosis, These patients were treated at a time when the importance of fractionation in whole body hyperthermia was not realised, and these patients died within one week of treatment. Similar tumours have not been treated by fractionated therapy.

5.88 With the advent of gradually increasing dosages of thermotherapy, the treatment became safe and the effects easy to predict. At the same time it was realised that the likelihood of tumour cure by heat alone was small. Regression was dependant on the core of a large tumour mass being nutritionally deprived and therefore thermosensitive. Decreasing blood pressure and thus tumour blood flow during treatment accentuates this effect, resulting in a central area of necrosis surrounded by a cuff of viable cells, as observed by Overgaard and Overgaard (1975).

5.89 At this time the degree minute was adopted as our unit of hyperthermic therapy. From the graph of tissue sensitivities approximately 20 hours at 42°C., equivalent to 2,400 centigrade degree minutes, 41°C. standard, in a single fraction, would be required for tumour cure in

a sensitive tumour. From the cortisol response we did not consider it was feasibly possible to carry out such a prolonged treatment. The number of fractions required to build up to this sort of dose would be large and the treatment impractical. It was therefore considered unethical to continue without the addition of chemotherapy to the regime. All patients treated since 1974 have received a preliminary fractionated course of hyperthermia alone, with a subsequent treatment giving combined hyperthermia and cytotoxic drugs. This sequence has produced greater remission and, unlike hyperthermia alone, the tumours do not recur in an insensitive form after three months.

- 5.90 Hyperthermia alone produced a remarkable sense of well being during periods of remission with relief of tumour induced pain. This is probably due to tumour shrinkage though hyperthermia has been shown to cause conduction defects in nerves, the finer 'C' fibres transmitting pain impulses being the most responsive (Young and Henneman, 1961). The great improvement which was observed in the quality of life possible after palliative treatment in a responsive tumour justified the treatment of such advanced cases.

PART TWO.

LOCAL HYPERTHERMIA.

SECTION 6.METHODOLOGY OF LOCAL
HYPERTHERMIA.

- A. PRINCIPLES OF LOCAL HYPERTHERMIA
- B. METHODS OF INDUCING LOCAL HYPERTHERMIA
- C. A NEW METHOD INVOLVING HYPERTHERMIC
PERFUSION OF THE DISTENDED URINARY
BLADDER UNDER EPIDURAL ANAESTHESIA.

A. PRINCIPLES OF LOCAL HYPERTHERMIA

6.1. Methods of temperature elevation in a part of a body can be divided into those in which the blood supply to the part is temporarily arrested and those in which the blood supply remains intact. The importance of the cooling effect of blood flowing through vasodilated hyperaemic tissue is reflected in the observation that the temperature recorded by a thermometer situated in tissue at a distance from a constant heat source rises to a maximum and then falls off to a steady reading. This fall in temperature is due to the increase in blood flow caused by reactive vasodilation (Rowell, Brengelman et al, 1970).

6.2 Moritz (1947) found that with a skin temperature of 45°C . for three hours the temperature recorded at 2 mm. deep was 44°C . There is therefore a large thermal gradient (0.5°C . per mm.), with poor heat penetration. Heat penetration may be improved by an increase in the applied temperature. However, at temperatures in excess of 44°C . there is little difference between the effect on tumour and normal tissues. 45°C . is the temperature at which the sensation of heat is transferred to that of pain in the human skin (Belehradek, 1957). This limits the maximum local

temperature, temperatures above 45°C. cause irreversible damage as a burn as opposed to heat stress and are painful. Such a limitation gives a poor penetration from local external heating extending over a few millimetres of tissue before ineffective temperatures are reached.

6.3 Heat penetration can be improved by inhibiting the blood supply to the part, by methods such as applying a tornequet to a leg. The problem is then complicated by ischaemia and time limitation. Technical aspects of perfusion of the isolated limb brings its own complications resulting from both the surgery involved perfusion pressures used.

6.4 In this part of the thesis the methods of inducing local hyperthermia are reviewed. A trial of local hyperthermic perfusion of the distended urinary bladder in the treatment of transitional cell carcinoma was carried out. It was hoped that by local pressure it would be possible to reduce the blood flow to the bladder sufficiently to allow heat penetration, and by exposing the whole tumour, enable curative therapy to be carried out.

B. METHODS OF INDUCING LOCAL
HYPERTHERMIA.

Short Wave Diathermy

- 6.5 In short wave diathermy, ultra high frequency electrical currents are passed through the body between two electrodes in contact with tissue. Because the current has a tendency to spread, its concentration per square centimeter diminishes as the distance from the electrode is increased (Schwan and Piersol, 1954). Heating is dependant on the resistance of the tissue through which the high frequency current passes. The resistance of tissue depends on the relative amounts of fat, water and electrolytes as well as cell size and shape, which together make up the dielectric properties of the tissue.
- 6.6 Fat and bone have very different dielectric properties, and the heat which is developed per volume unit of subcutaneous fat is greater than that produced in deep tissues which have a higher water content (Johnson and Guy, 1972). This difference becomes less as the frequency is increased but even at the highest practical frequency of about 100 Mc., fatty tissue is heated more than muscle tissue (Schwan and Piersol, 1954).

6.7 Short wave diathermy was extensively used in the treatment of gonorrhoea prior to the advent of penicillin. Using a rectal, water cooled, electrode and broad abdominal pads, Herring (1953) reported achieving a prostatic temperature of 46°C . Percy (1916) used local diathermy at an open laparotomy to raise the temperature of inoperable uterine carcinomas to 45°C . with good results. However, no histological confirmation was given in his reported series.

Heating by Probes Inserted into the Tumour

6.8 Heated probes suffer from an extreme limitation of heat penetration. Single probes have been used with limited success to potentiate chemotherapy in the treatment of gliomas of the brain (Sutton, 1971). Doss (1975), implanted parallel needles at 1 cm. intervals as a planar implant, and then produced eddy currents by electromagnetic induction so heating the tissue between the needles. Following eight daily treatments of one hour at 45°C . he was able to produce remarkable regressions, and a cure rate in both animal and human squamous cancers recurrent after previous conventional therapy.

Electromagnetic Heating

6.9 Using circulatory high frequency currents a changing magnetic field can be induced within the tissue. The strength of the electromagnetic field so induced is proportional to the current passing. Heating is induced by eddy currents formed by changing magnetic field, and is therefore dependant on the frequency. Penetration is influenced only by the magnetic properties of the tissue and these do not vary within the human body (Schwan and Piersol, 1954). Using large circulatory currents at 27 megahertz good penetration is possible but with the available equipment the energy imparted would not be sufficient to raise the thigh above 39°C . (Guy, 1975). The equipment necessary to induce greater heating is very expensive but is theoretically possible. Lower levels have been used in the treatment of lymph nodes spread by injecting a target material into the lymphatics (Gilchrist, 1965).

Microwave Heating

6.10 Microwaves are produced in a magnetron by accelerating electrons in a spiral orbit. The frequency of rotation of the electrons is related to the wave length of the electromagnetic wave produced. The effects of electro-

magnetic field produced as a wave passes through the tissue are due to oscillation of the free charges of ions and rotation of dipole molecules at the frequency of the applied electromagnetic energy (Johnson and Guy, 1972).

6.11 Microwaves are reflected at tissue interfaces, such as fat/muscle or muscle/bone, producing 'standing waves' by reflection. Those 'hot' spots in the tissues with the lower dielectric constants can be decreased by using lower frequencies, than the standard 2450 megahertz, with a longer wave length. This also gives an increased penetration (Johnson and Guy, 1972). Even at 750 megahertz there is scatter of microwaves by tissue and also focusing of microwaves by round structures producing hot spots.

6.12 Considerable use of microwaves has been made in the treatment of human cancer, the most recent of which is by Holt (1975). Some success has been claimed for this method of treatment, however, it has been used in conjunction with other forms of therapy and no actual tumour temperatures have been given. This is mainly because of the difficulty of measuring temperature in a microwave beam as any metallic objects are heated. Recently crystal thermometers have been invented which may be of

use in this field. Effects of hyperthermia in these cases are difficult to assess without knowing the temperatures induced.

Heating by Ultrasound

- 6.13 Ultrasound is produced by very rapid oscillation of a diaphragm. The sound waves so produced are conducted through tissue and generate heat by vibration of the tissue through which they pass. There is preferential heating of bone and bone marrow (Nelson, Herrick and Krussen, 1950), and the temperature of nerves rises to approximately twice that of the surrounding tissue (Herrick 1953). Of particular importance is reflection from interfaces with hollow air-filled organs and this severely limits the use of ultrasound.
- 6.14 It may have a particular use in the treatment of brain tumours in that it can be well localised, and there is an increased production of heat in the tumour as compared with the surrounding brain tissue (Lele, 1975). For this procedure burr holes are required to remove the bone prior to application of the ultrasound beams. In other tissues distribution and scatter appears too great to make it useful in its present form.

Vascular Perfusions with Heated Solutions

6.15 Hyperthermic perfusion of the hind limbs of guinea pigs and cats had been carried out by Kellaway and Rawlinson, 1944, who found that damage occurred at temperatures above 45°C . and 43°C . respectively. Cavaliere, Ciocatto et al, (1967), studied hyperthermic perfusion on the dog's hind limb, and found that temperatures of between 42 and 44°C . could be tolerated for two hours without major damage. They then used the same technique on human tumour bearing limbs. In 22 patients with cancer of limbs, the temperatures of the tumours was raised to between 41.5 and 43.5°C ., for several hours. In 25 regional perfusions with pre-warmed blood Cavaliere, Ciocatto, et al (1967). Nine further patients were treated but severe complications led to six deaths and three immediate amputations. In ten of the surviving patients the tumours completely disappeared, however, three subsequently recurred. Three patients failed to respond, four could not be evaluated, and in five there was evidence of tumour regression. Cavaliere's method involved cannulating the main artery and vein but did not isolate the limb from the general circulation, and modifications had to take into account toxic products absorbed during treatment.

6.16 In 1958 Creech, Krementz, et al reported

their experience of perfusion of tumour bearing limbs, isolated from the rest of the body by tourniquet, with high doses of Phenylalanine mustard. The rationale was to deliver a high dose of the cytotoxic agent directly to the tumour and its venous and lymphatic drainage, in a dose which would be fatal if administered systemically.

6.17 Stehlin, Giovalella et al (1975) combined the two ideas, and, by isolating the limb were able to perfuse it with a heated isotonic solution of melphalan. He has produced remarkable regressions in melanoma treated in this way. The advantages of this type of perfusion are that as blood is not used there is no need for systemic anti-coagulants. The red blood cells are not broken down as they could be using a combination of high dose chemotherapy plus heat, and high dose chemotherapy and hyperthermia can be used safely.

6.18 The disadvantages are in the complexity of the method, requiring a skilled surgeon to carry out the isolation of artery and vein. It is necessary to have some pressure to drive the circulation but at ordinary arterial pressure there is congestion and development of a 'sick limb' (Stehlin, 1975). The inflow pressure has to be adjusted so the inflow and outflow rates are exactly the same. The other problem which

may be important is that, as with coronary bypass, pulsatile flow is necessary for bypassing more than two hours, Dalton, McCarty, et al, (1965). It may be that pulsatile flow would decrease the complication rate in local perfusion. Koops (1972) has suggested that post perfusion oedema induces ischaemia and recommends fasciotomy as a routine decompression procedure prior to treatment.

C. A NEW METHOD INVOLVING HYPERTHERMIC
PERFUSION OF THE DISTENDED URINARY
BLADDER UNDER EPIDURAL ANAESTHESIA.

Introduction

6.19 Previous methods of perfusion of the urinary bladder did not involve distention. It was thought that in the contracted state some of the tumour would be shielded from the perfusate by folds of normal bladder. This together with other factors discussed later in the thesis prompted the present study. The trial was carried out to assess the effect of hyperthermic perfusion on the distended urinary bladder and the tumour it contained.

6.20 All patients in this series had a biopsy proven transitional cell carcinoma of the urinary bladder, recurrent following radiotherapy, and not controlled by cystodiathermy. Tumours were Stage III, being palpable on bimanual examination and had spread through the whole thickness of the bladder wall into the surrounding structures. Most presented with gross haematuria, not controlled by repeated cystodiathermy. One patient was treated for gross haematuria due to post radiotherapy telangiectasia.

6.21 Initially, single treatments of increasing duration were given. Subsequently, fractionated treatments were used and a comparison made between the effects of perfusion with and without

epidural anaesthesia. A total of 115 treatment sessions in 28 patients have been carried out.

6.22 This section has not been divided into material and methods followed by results as each series was based on findings of the previous study. The method used is basically the same throughout but reasons for the modifications and these modifications are given at the beginning of each study.

Method

6.23 It was found that even slight bladder distention proved uncomfortable, and, because of the variation in flow rate, caused by intermittent bladder contraction, the outflow temperature was difficult to control. Continuous epidural anaesthesia allowed relaxation of the bladder and a steady flow rate to be achieved at a constant outflow temperature. Epidural anaesthesia was achieved by introducing an epidural catheter at the L4/5 level and feeding it cranially for approximately two inches. Anaesthesia was induced by intermittent injections of 1.5% plain Xylocaine to achieve anaesthesia up to the level of T10.

6.24 Under epidural anaesthesia the nature and extent of the tumour was established by cyst-

oscopy and bimanual examination. Biopsies were taken from the tumour and from the normal mucosa. The bladder capacity was measured, and a 24F three-way Foley catheter introduced into the bladder and inflated with 5 ccs. of warm saline. The bladder was then washed out to remove air bubbles or clots and perfused with normal saline, heated by passage through a heating coil which was inserted in the water bath.

5.25 The outflow from the catheter ascended over a gradient to an overflow tank (Figure 40). The height of the overflow tank was adjusted so that slightly less than the known bladder capacity caused overflow. This was usually at a height between 15 to 20 cms. above the symphysis pubis. Patients were placed on an X-ray table during treatment, and the degree of bladder distention was monitored radiographically by injecting a coloured solution of radio-opaque medium into the perfusate and taking check films (Figure 41).

6.26 Thermocouples were used to measure inflow, outflow and rectal temperatures. A further probe was attached to the skin, one inch above the symphysis pubis. In three patients a further probe was inserted into the tumour cystoscopically.

- 6.27 Perfusion was at a constant rate of 3 litres per hour, the inflow temperature being adjusted to give the required outflow temperature of 45°C . (Hall et al, 1974), but this caused lower abdominal discomfort and the temperature was therefore reduced to 44°C . which was well tolerated by all patients (Figure 42). Three patients were treated at an outflow temperature of 43°C . for four hours.
- 6.28 At the beginning of the perfusion there was a rapid rise in outflow temperature which remained stable providing the inflow temperature was kept constant (Figure 43). The rise in skin and rectal temperatures depended to some extent on the size of the tumour. It was greatest when the tumours were infiltrating and extensive, rising to 40.5°C . in such cases during the first two hours of treatment.
- 6.29 At the end of the treatment session a Foley catheter was inserted and left on free drainage for 24 hours before removal. The effect of treatment was assessed by cystoscopy, bimanual examination and biopsies. These were initially taken three days and two weeks after treatment, and thereafter at six weekly intervals. In later cases biopsies were also taken immediately at the end of treatment. At each assessment a

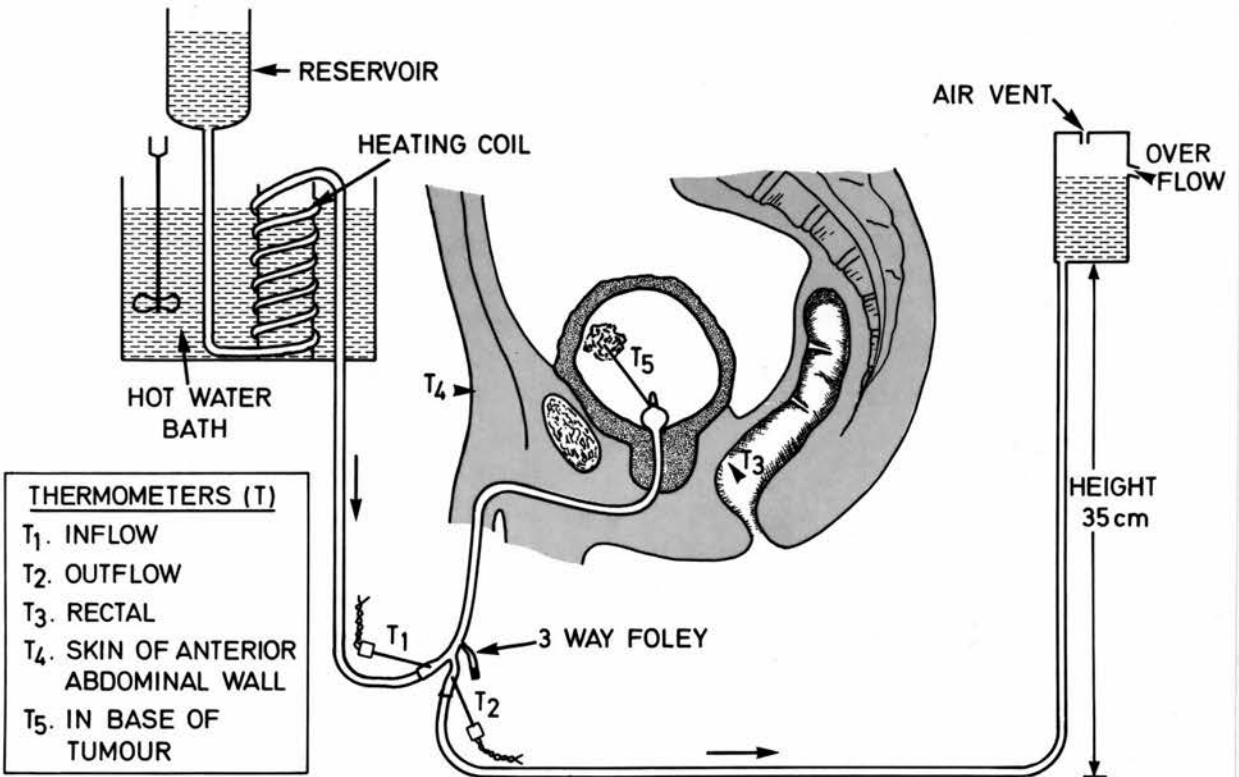


FIGURE 40.

Method of hyperthermic perfusion of the distended urinary bladder. The overflow height was approximately 35 cms. above the table or 15 - 20 cms. above the symphysis pubis.



FIGURE 41.

Radiograph during hyperthermic perfusion showing distention of the bladder and minimal reflux in the left side.

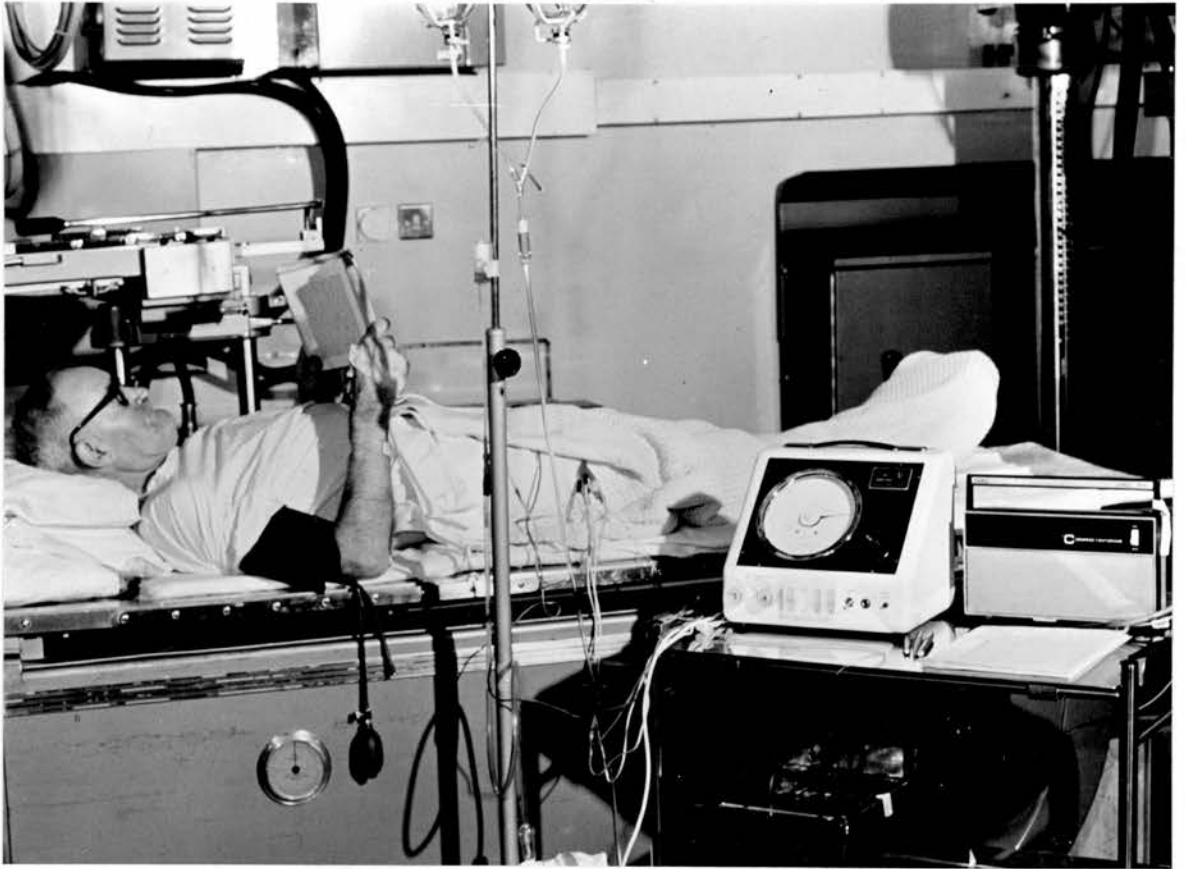


FIGURE 42.

A patient during hyperthermic perfusion of the distended urinary bladder. The patient is lying on an X-ray table to enable check films of bladder distention to be taken.

HYPERTHERMIC PERFUSION OF URINARY BLADDER

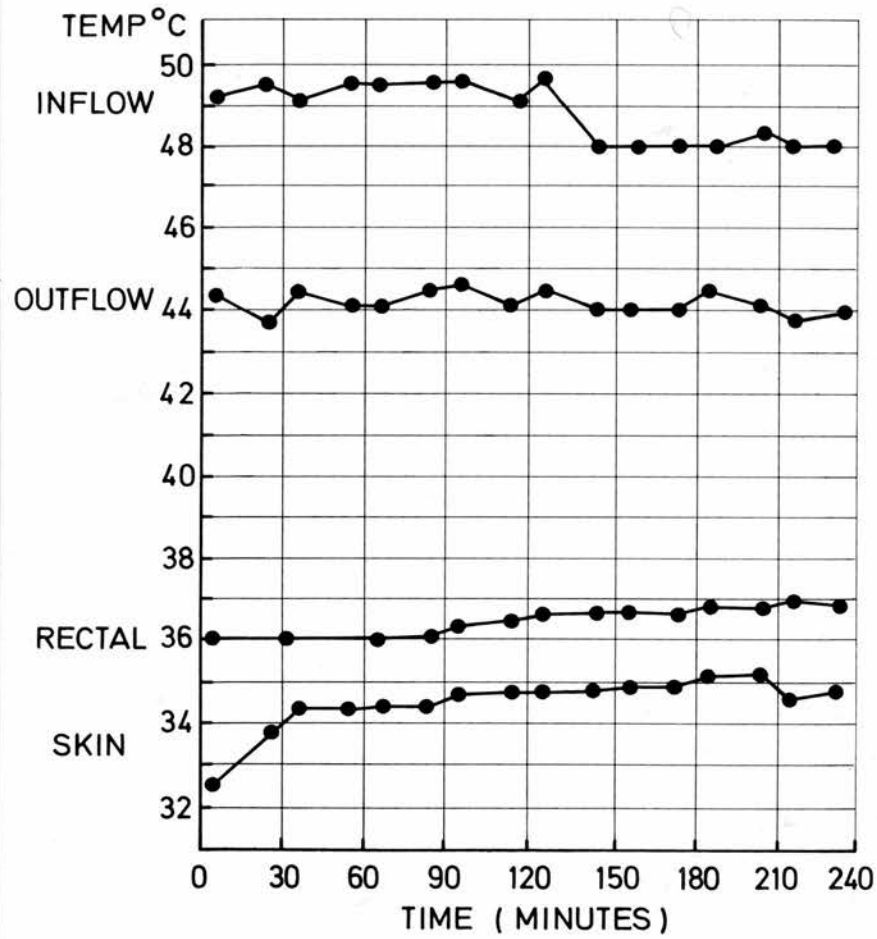


FIGURE 43.

Temperature graph during hyperthermic perfusion showing a stable outflow temperature is maintained.

a special assessment sheet was completed without referral to previous assessment sheets, and a drawing of the appearances of the bladder made (Figure 44).

Homogeneity of Heating

6.30 In three cases a probe was inserted into the tumour cystoscopically. However, when the bladder emptied as the cystoscope was withdrawn the probe cable was forced down the urethra. When the bladder was distended during treatment, the probe pulled out of the tumour. The reading on the probe was similar to that of the outflow and at cystoscopy following treatment the probe was found to be lying freely in the bladder. It is therefore not known when the outflow temperature accurately reflects the tumour temperature.

6.31 It was not possible to calculate this treatment in terms of centigrade degree minutes as there was a temperature gradient across the tumour. Treatment was therefore recorded in terms of outflow temperature and duration of perfusion.

6.32 As there is also a possibility of incomplete circulation of the perfusate in that the heated inflow may be circulating out

BLADDER ASSESMENT

Name _____

Unit No. _____

Date _____

Date of Birth _____

Weight _____

CYSTOSCOPYResidual Volume ccs. Capacity ml.
 Papilloma ☐ ☐ ☐
 Solid ☐ ☐ ☐
 Ulcerative ☐ ☐ ☐

 Single ☐
 2-5 ☐
 10 ☐

 Satallites ☐
 6-10 ☐
 Size cm.
SITE
 Trigone (L) ☐
 Lat wall (L) ☐

 Trigone (R) ☐
 Lat wall (R) ☐

 Ant wall ☐
 Post wall ☐

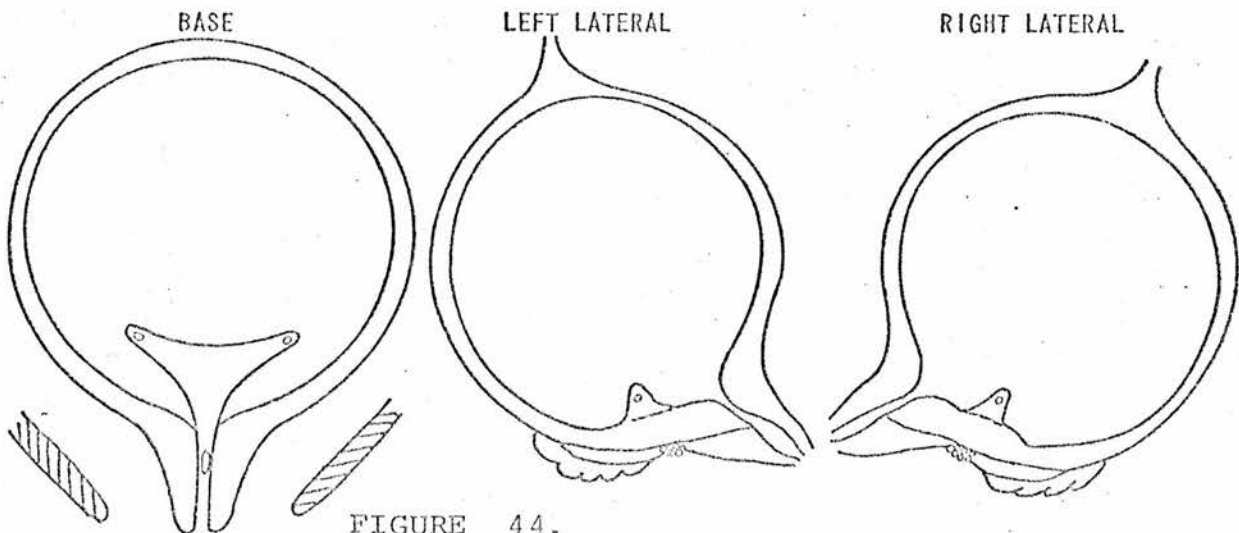
 Vault ☐
 Urethra ☐
BIMANUAL ASSESSMENTSize cms. Impalpable ☐Induration superficial ☐Induration deep ☐Extra vesical mobile mass ☐Fixed mass ☐Nodes ☐BIOPSYBenign ☐Transitional cell Ca. well differentiated ☐" " " " moderate diff. ☐" " " " anaplastic ☐S.C.C. ☐Adeno Ca. ☐Other ☐IVP.Normal ☐Abnormal ☐LYMPHOGRAMNormal ☐Abnormal ☐

FIGURE 44.

This sheet was completed at each cystoscopy. The position of tumour was marked, if at the vault the patient was treated on his side. Biopsy sites were also marked.

through the outflow hole of the Foley catheter without circulating round the bladder, a plastic bag was used to represent the bladder and dye put into the infusate (Figure 45). Good circulation of the infusate round the bladder was found in repeated tests.

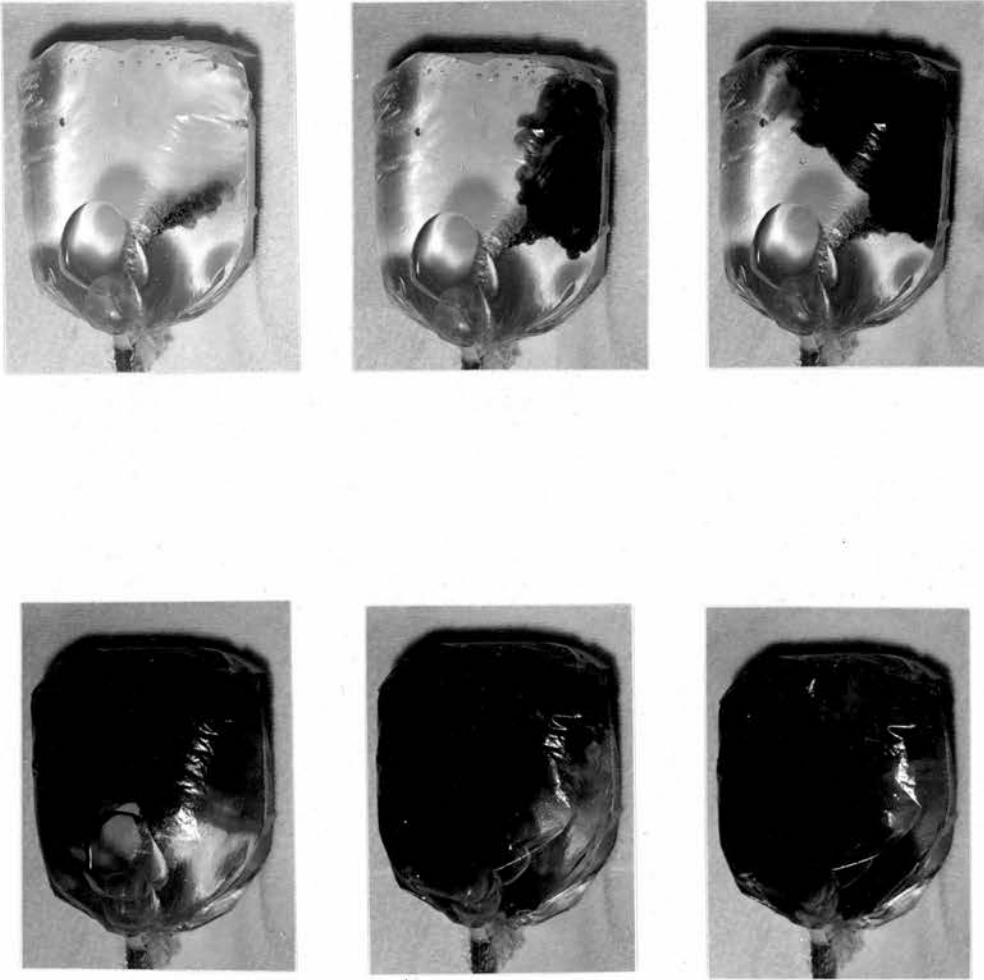


FIGURE 45.

Serial photographs showing circulation of dye during perfusion, under the same conditions as used in the human bladder, showing good mixing of infusate.

SECTION 7.

STUDIES CARRIED OUT TO
ASSESS THE SENSITIVITY
OF TUMOUR AND THE NORMAL
BLADDER TO HYPERTHERMIA.

- A. SINGLE TREATMENTS OF INCREASING DURATION
UNDER EPIDURAL ANAESTHESIA.
- B. FRACTIONATED PERFUSION UNDER EPIDURAL
ANAESTHESIA.
- C. FRACTIONATED PERFUSION WITHOUT EPIDURAL
ANAESTHESIA.
- D. ELECTRON MICROSCOPIC AND THYMIDINE
UPTAKE STUDIES.
- E. SINGLE TREATMENTS UNDER EPIDURAL
ANAESTHESIA FOR THE ARREST OF GROSS
HAEMATURIA.

A. SINGLE TREATMENTS OF INCREASING
DURATION UNDER EPIDURAL ANAESTHESIA

- 7.1 Using the method described, patients were treated by gradually increasing durations of treatment at an outflow temperature of 44°C . Fifteen treatments were carried out in thirteen patients, and the duration of perfusion varied from 1 to 4 hours. In all of the initial patients, gross haematuria was arrested but slight haematuria persisted for up to one week following treatment.
- 7.2 After treatment of one hours duration there was no frequency. At cystoscopy the mucosa appeared healthy and the bladder capacity was not altered, there was however, residual viable tumour present. These two patients had recurrent haematuria in six weeks and required further treatment (Figure 46).
- 7.3 After three hours at 44°C . there was obvious tumour necrosis appearing at cystoscopy as a grey-white slough which took up to two months to separate from the mucosa. The normal mucosa appeared red and inflamed and the bladder capacity was reduced to between 150 - 200 mls. There was no evidence of recurrent tumour for three months, thereafter recurrent tumour was found in all patients.
- 7.4 After four hours at 44°C . there was

LOCAL BLADDER HYPERTHERMIA

COMPARISON OF CLINICAL RESULTS FOLLOWING DIFFERENT TEMPERATURE /
TIME COMBINATIONS

OUTFLOW TEMP. °C	TREATMENT TIME HOURS	DURATION OF FREQUENCY WEEKS	EFFECT ON TUMOUR
44	1	NIL	NIL
44	2	1	NIL
44	3	12	Clear 3/12
44	4	20 +	Clear 5/12 +

43	4	6	SOME NECROSIS

FIGURE 46.

The effects of different durations of perfusion at
an outflow temperature of 44°C.

prolonged and continuing frequency and at cystoscopy the bladder capacity was reduced to between 50 and 75 mls. The whole of the bladder mucosa was covered with a grey-white slough. Bimanual examination revealed an indurated palpable bladder. Because of continued frequency within this group urinary diversions became necessary. Figure 47 shows calcification in the fibrosed wall of the bladder of one such patient. He remained fit and well with no evidence of recurrent tumour at 2 years post treatment.

- 7.5 Patients treated at an outflow temperature of 43°C . for four hours had frequency and cystoscopic findings equivalent to those treated at 44°C . for two hours. The latter are recorded along with those treated for gross haematuria by treatment at 44°C . for two hours in a separate section.

Histopathological Changes

- 7.6 The tumour biopsies taken before hyperthermia were graded as described by Bergkvist (1965). Six tumours were of Grade II, one of which was invasive, six were Grade III, five of which were invasive and one was Grade IV and invasive. The effects of hyperthermia on the bladder vary according to the interval after treatment.

- 7.7 Changes seen in the transitional cell at the end of treatment session consisted of nuclear vacuolation, pyknosis, with vacuolation of the cytoplasm and reduction of the epithelial adhesion. These changes were similar to those reported in the epidermis after thermal injury (Moritz, 1947). Slight changes were not easy to assess in a bladder which was inflamed following hyperthermia and had previously been subjected to radiotherapy and repeated cystodiathermy. Severe effects were easily recognised in both tumour and mucosal epithelium and were often accompanied by vascular connective tissue changes.

Effects of Hyperthermia on Bladder Tumour

- 7.8 No tissue necrosis was seen in tumour biopsies taken after one hours perfusion at 44°C. The effects seen in biopsies taken immediately at the end of the 3 - 4 hours perfusion were often patchy. One part of the tumour showing little change whilst another showed complete necrosis.
- 7.9 Necrotic areas were accompanied by local vascular changes such as necrosis and thrombosis in the small vessels of the tumour and haemorrhage into its stroma. These vascular changes may be



FIGURE 47.

Plain radiograph of a patients pelvis six months after hyperthermic perfusion at an outflow temperature of 44°C . for four hours. The bladder is outlined by phosphatic debris lining the walls.

responsible for some of the tumour destruction since blood vessels are more susceptible to thermal injury than is normal epithelium (Moritz, 1947).

7.10 Necrosis manifested itself in two main forms, either a ghost-like villi or in distintegration of the tumour with disruption of its cells (Figure 48). Three days after perfusion for three to four hours, necrosis was usually so advanced that it was difficult to recognise tumour in the biopsy material.

7.11 Three of the tumours recurred after two months or more and were of the same Bergkvist (1965) grade as prior to treatment.

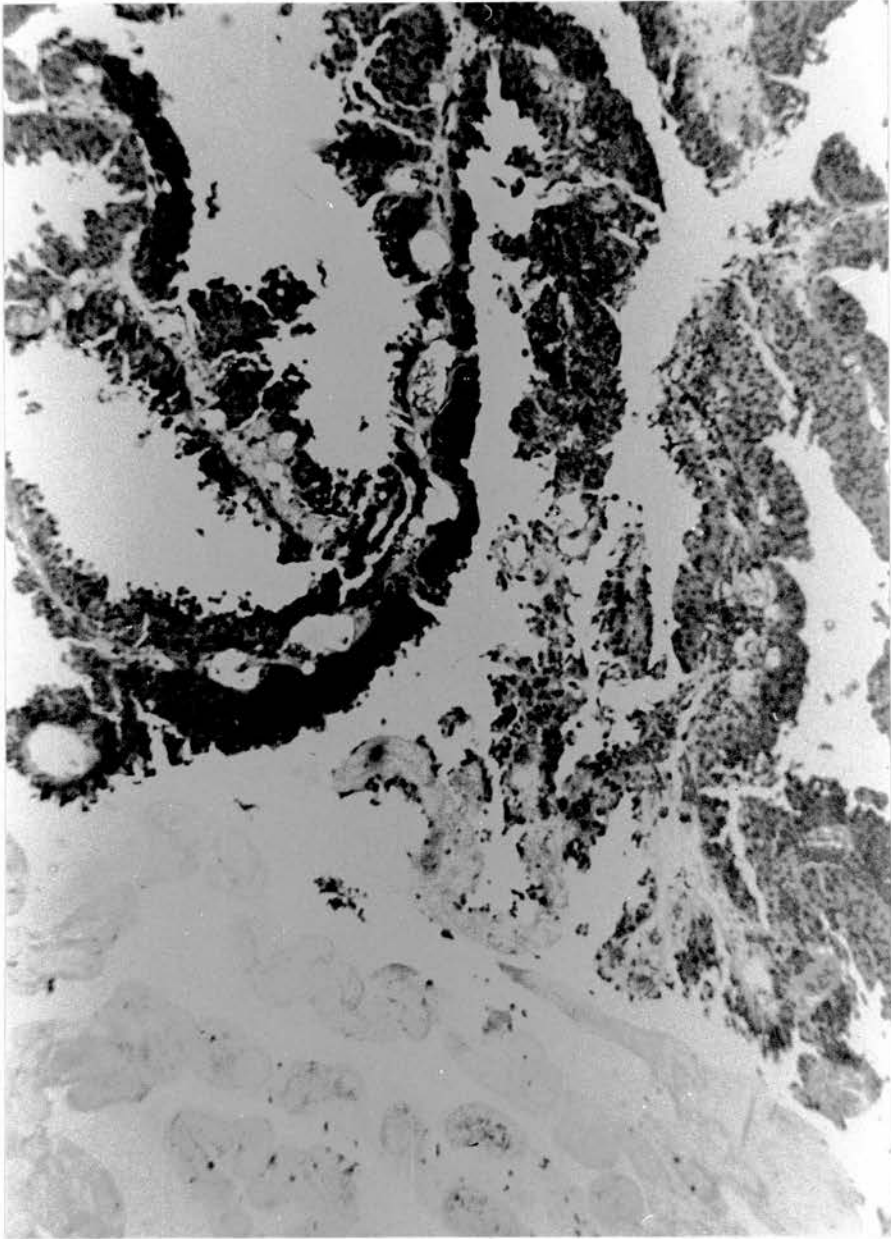


FIGURE 48.

Biopsy of transitional cell carcinoma immediately after perfusion for three hours at an outflow temperature of 44°C . Some villae in the lower aspect of the illustration are ghost like whereas some of the deeper layers appear normal.

Effect of Hyperthermia on Bladder Mucosa

- 7.12 The normal mucosal epithelium initially was considerably less affected than the tumour. Quite marked changes however, occurred in the blood vessels and connective tissue of the mucosa and these led to additional changes in the epithelium. The depth to which these vascular changes were visible was related to the duration of the hyperthermic perfusion.
- 7.13 Hyperthermia resulted in congestion and oedema of the lamina propria with an inflammatory cell infiltrate sometimes including numerous eosinophils. Thermal effects upon blood vessels of the mucosa led to exudation of plasma and occasionally to interstitial haemorrhage. Some of the vessel walls were frankly necrotic (Figure 49), and thrombi tended to form (Figure 50).
- 7.14 These vascular changes exacerbated the direct thermal injury of the lamina propria, sometimes resulting in areas of necrosis with exfoliation of epithelium and subsequent formation of hypervascular inflamed granulation tissue, and a degree of fibrosis usually followed (Figure 51). Regeneration of epithelium was obvious in all biopsies taken two months after hyperthermia but may have been established sooner.

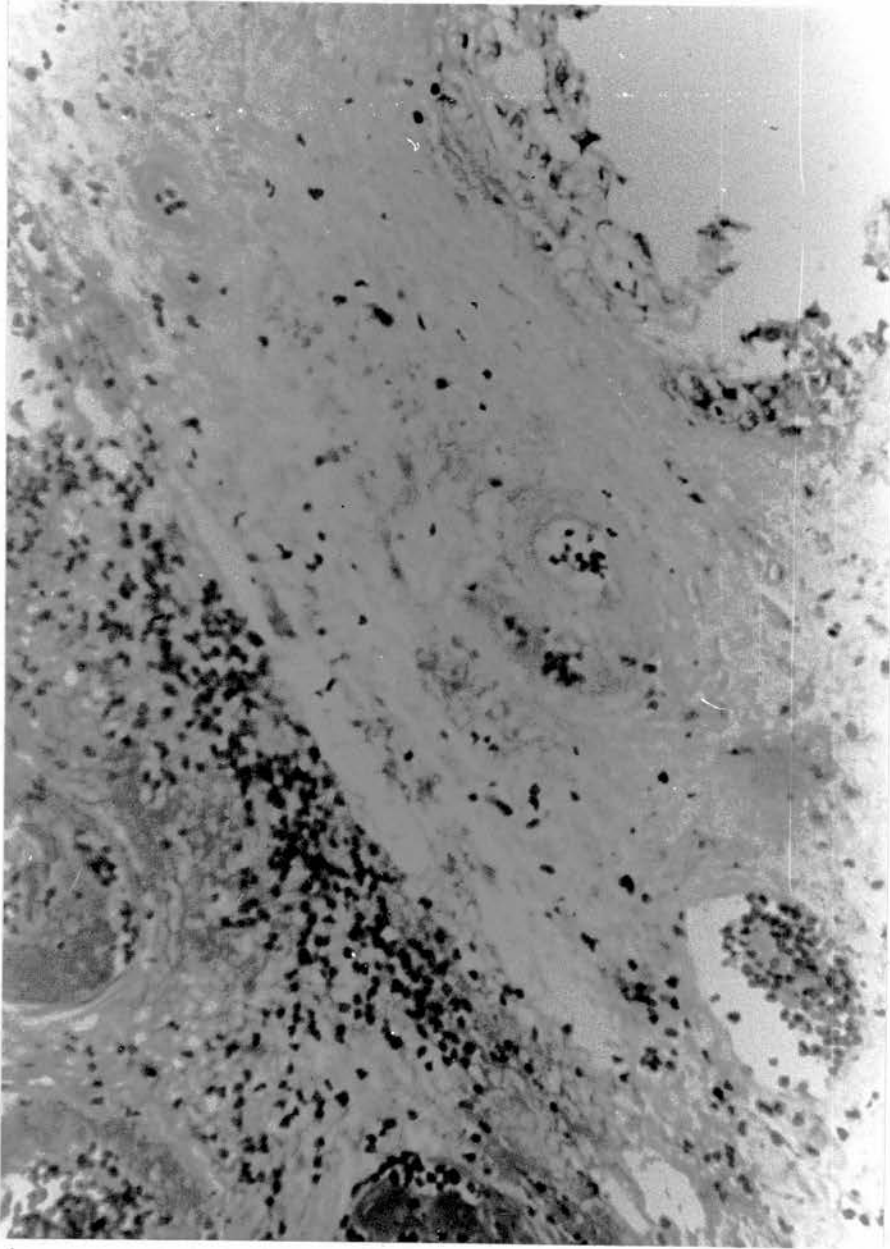


FIGURE 49.

Bladder wall three days after perfusion at 44°C. for four hours showing vascular necrosis and haemorrhage into the stroma with loss of the epithelium.

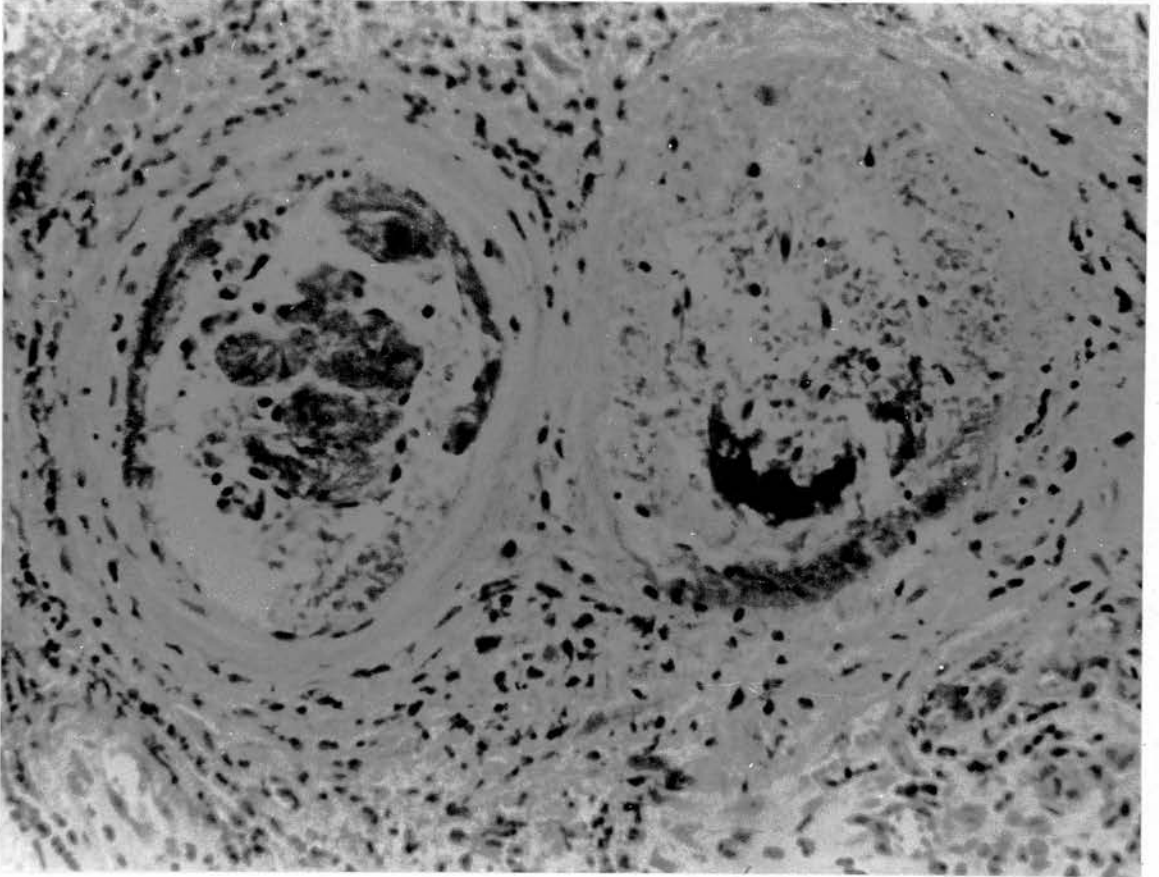


FIGURE 50.

High power view of a stromal vessel showing thrombosis in arteriole and venule three days after perfusion at 44°C. for one hour.

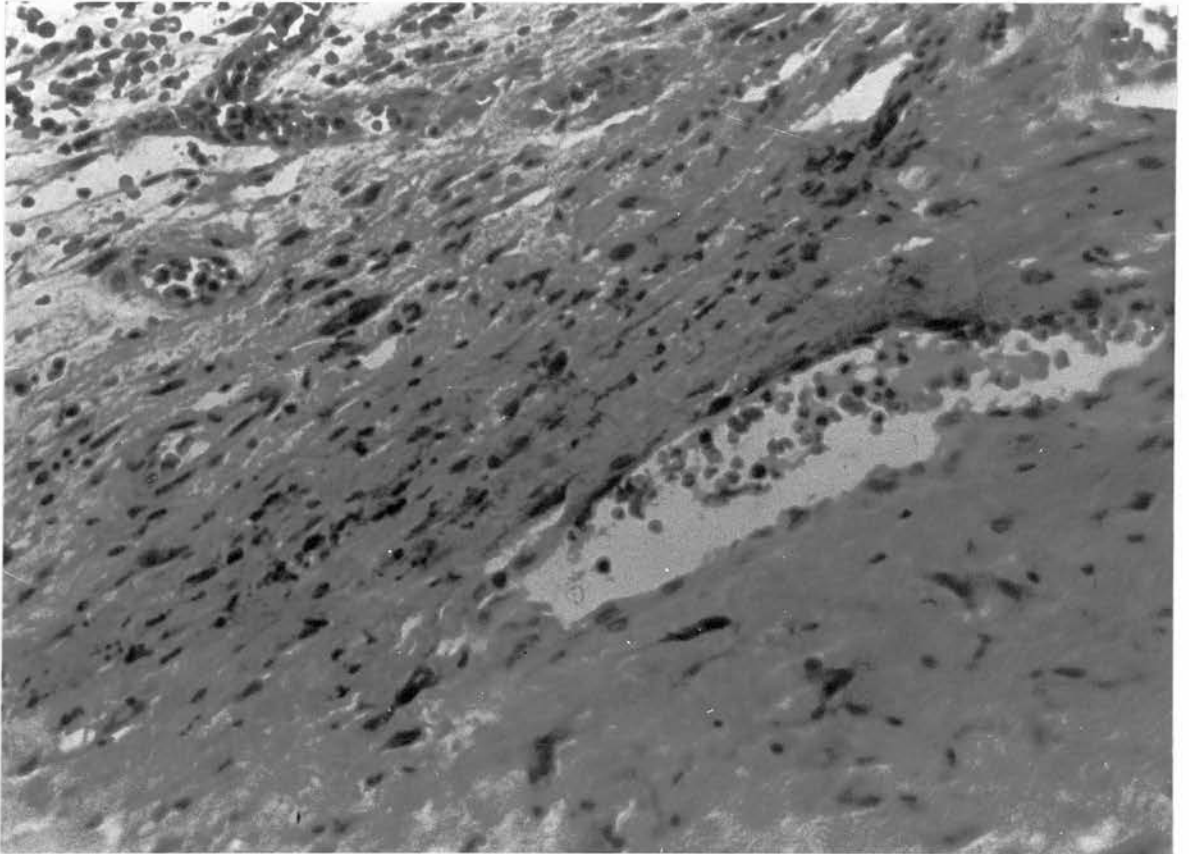


FIGURE 51.

Biopsy of the superficial muscle layer of the bladder wall two months post perfusion at 44° C. for four hours. This shows disorganisation and fibrosis of the muscle coat with a diffuse inflammatory infiltrate still present. The mucosa had entirely regenerated but the patient had marked frequency with a bladder capacity under anaesthesia of 50 cc.

B. FRACTIONATED PERFUSION UNDER EPIDURAL
ANAESTHESIA.

7.15 From the previous studies it appeared that under epidural anaesthesia that normal bladder tolerance to hyperthermia at an outflow temperature of 44°C . was one hour. However, from previous studies on tumour and normal cell sensitivities, assuming the whole tumour was at 44°C ., the time for total tumour destruction would be approximately five hours. From Thymidine uptake studies it appeared that 17 hours was required before the mucosa cells fully recovered from a hyperthermic episode. Fractionation was therefore done on a daily basis. Initially, for five single treatments each lasting one hour.

Method

7.16 The method used was as previously described, the epidural catheter was left in situ over the five days of treatment and local anaesthesia induced by injection of 1.5% Lignocaine. After four daily treatments the patients were cystoscoped and the tumour and bladder assessed prior to giving further hyperthermia. Three patients were managed in this way, the first had a total of 5 , one hour treatments, the second, 4 hours 40 minutes in four fractions followed by a 2 hour fifth treatment. The third had 5 hours in four fractions followed by

a fifth single fraction of two hours.

Results

7.17 At cystoscopy on the fourth day following four single fractions of at least one hour there was a slight decrease in bladder capacity, the normal mucosa appeared healthy and the tumour obviously viable.

7.18 Following the fifth fraction of two hours in the latter two cases there was some tumour necrosis appearing as white slough. Following treatment the two patients who had a single fraction of two hours at the end of their treatment had slight frequency lasting for one week.

Histology

7.19 There was extensive superficial necrosis of the tumour, the tips of some of the villae were totally necrotic and in other areas minute vessels contained thrombi with haemorrhage into the stroma. Most of the deeper portion of the tumour did not show these changes and the basal portions appeared relatively unaffected by hyperthermia.

7.20 The 'normal' bladder wall showed inflammatory congestion, oedema and infiltration by inflammatory cells including eosinophils. No vascular necrosis could be seen and the muscularis appeared normal.

The charges were similar to those occurring after a single fraction of two hours at an outflow temperature of 44°C.

C. FRACTIONATED PERFUSION WITHOUT EPIDURAL ANAESTHESIA.

7.21 Hyperthermic perfusion of the urinary bladder without epidural anaesthesia is a simple procedure which, after suitable instruction, can be carried out by nursing staff. Five of the thirteen cases treated in this way were managed mainly by the nursing staff, under supervision.

7.22 Following the initial studies it was thought that part of the problem of normal bladder damage was due to the fact that patients had previously had a radical course of X-ray therapy (5,500 - 5,750 rads. to max in 20 fractions over four weeks). Hall (1976) has shown by contrast radiography that the bladder can be distended and perfused without epidural blockade, and he had been able to perfuse the bladder for periods in excess of five hours as a single treatment without subsequent side effects. The patients he perfused had not received previous radiotherapy and in order to assess this possibility a series of patients were treated without epidural blockade.

Method

7.22 The method used was the same as previously described but the height of the overflow tank had to be decreased to a maximum of five inches suprapubically because of pain. All patients were premedicated with Omnopon and Probanthine to try and prevent bladder spasm. Initially, patients were treated with five daily one hour fractions, at an outflow temperature of 44°C. This was gradually increased to five daily fractions of two hours at the same outflow temperature. At the end of the treatment session the patient was cystoscoped under general anaesthesia and biopsies were taken of normal mucosa and tumour.

Results

7.23 Patients tolerated this treatment extremely well and were in little or no discomfort during treatment as long as the overflow tank was not raised. There was no frequency or side effects following therapy. At cystscopy following treatment the bladder was only minimally inflamed and of the same capacity as pre treatment. The tumour did not appear to be affected and haematuria when present was not arrested.

Histology

7.24 There was little change in the pre or post treatment biopsies. In post treatment biopsies the muscularis appeared normal and the mucosa was covered by transitional cell epithelium which showed no sign of necrosis or even lesser thermal damage. The collagen of the lamina propria appeared swollen in some cases but this may have been due to trauma of biopsy. There was no damage to the larger blood vessels in any of the biopsies taken, and no evidence of tumour necrosis was observed.

D. ELECTRON MICROSCOPIC AND THYMIDINE UPTAKE STUDIES.

7.25 As a parallel study to the clinical findings and to evaluate the importance of local ischaemia, the normal epithelium and tumour were studied using electron microscopic and thymidine uptake techniques. Information on the ultra-structure and histo-chemistry of the human bladder is very limited (Brattifora, Eisenstein and McDonald, 1964). The study was therefore limited to changes which occurred in biopsies taken before and after treatment and to in vitro tissue slice work on these biopsies.

Method

7.26 The bladder was filled with saline at body temperature, rather than water at cystoscopy, and immediately the biopsy was taken it was divided in half. Half was sent for routine histology the other half was transferred quickly into Medium 199 (Gibco, Bio-cult). Sections were then cut for fixation for electron microscopic study, or thin tissue slices exposed for varying time intervals at 44°C. then incubated with C¹⁴ Thymidine for one hour. Excess Thymidine was then washed off, and the tissues fixed. A layer of photographic emulsion was poured over the section and auto radiographs obtained.

Results

7.27 In vivo and in vitro incubation at 44°C. for four hours caused tumour necrosis with loss of intracellular constituents and lysis of lysosomes (Figure 52,53). The normal mucosa both in vivo and in vitro appeared resistant to this degree of hyperthermia. To further study the effect on the vascular epithelium thin tissue slices from the deeper parts of the biopsies were incubated with Thymidine. There was no inhibition of Thymidine uptake by the vascular epithelium after four hours

exposure at 44°C. (Figure 53).

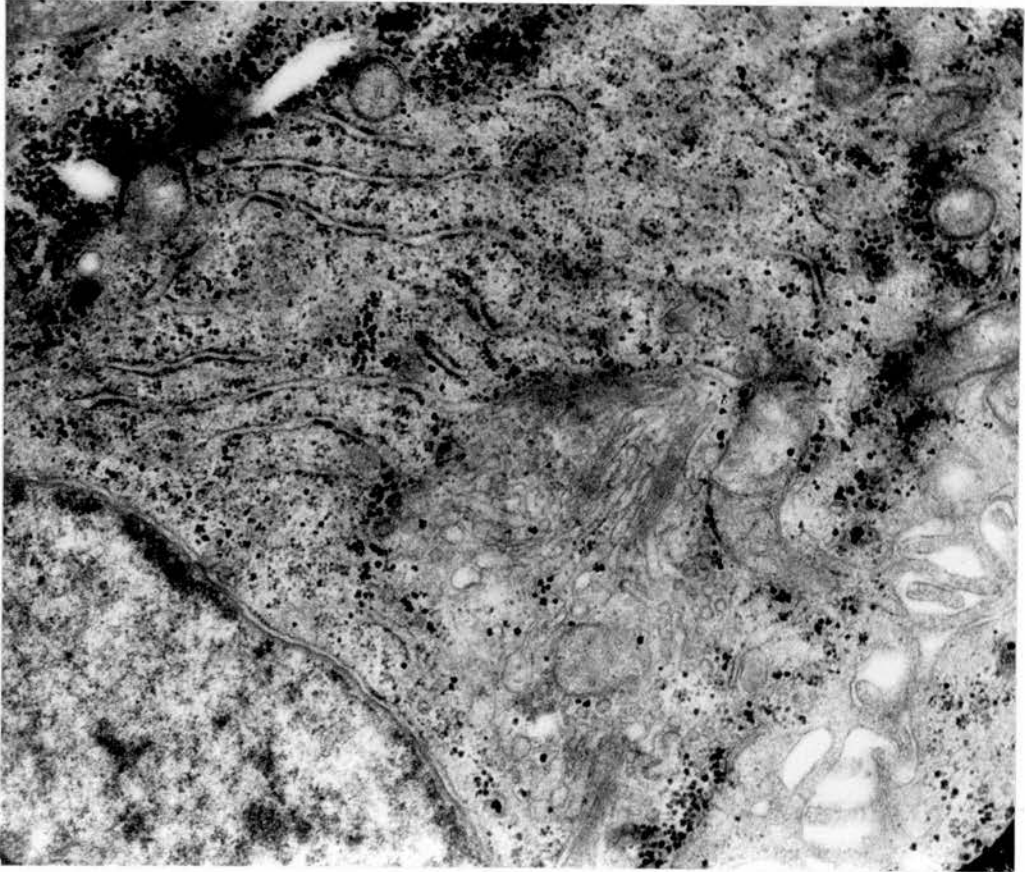


FIGURE 52.

Electron micrograph of part of a cell of a transitional cell carcinoma from a biopsy taken immediately before perfusion at 44°C. It shows a cell formed endoplasmic reticulum and nuclear membrane. Similarly the mitochondrial membrane and cristae are well formed and few lysosomes are present.

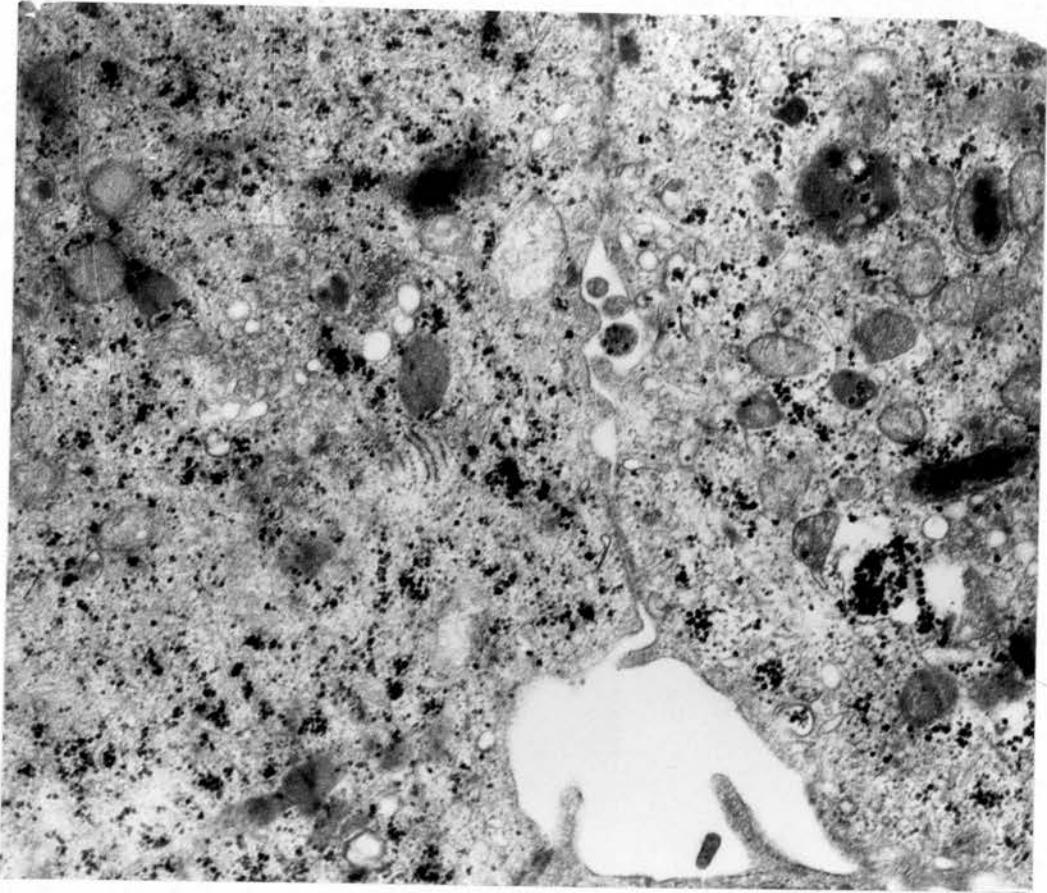


FIGURE 53.

Biopsy of transitional cell carcinoma taken immediately after perfusion at 44°C. for three hours. There is a loss of definition of the endoplasmic reticulum, the mitochondria are swollen and disorganised and numerous large lysosomes have appeared. The black specks are glycogen granules. These changes were also found after incubation of transitional cell carcinoma at 44°C. but not in the normal mucosal cells so treated.

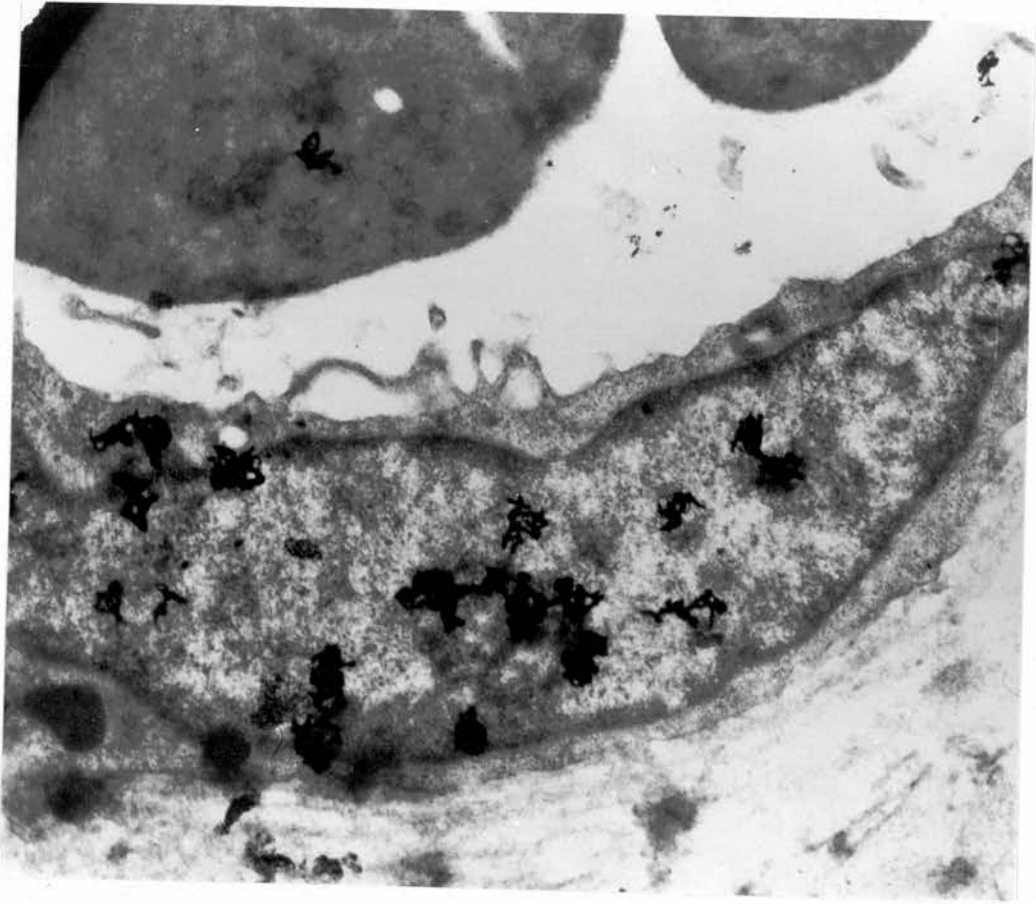


FIGURE 54.

Thymidine uptake autoradiograph of a vascular endothelial cell from a stromal arteriole incubated at 37°C. for one hour.

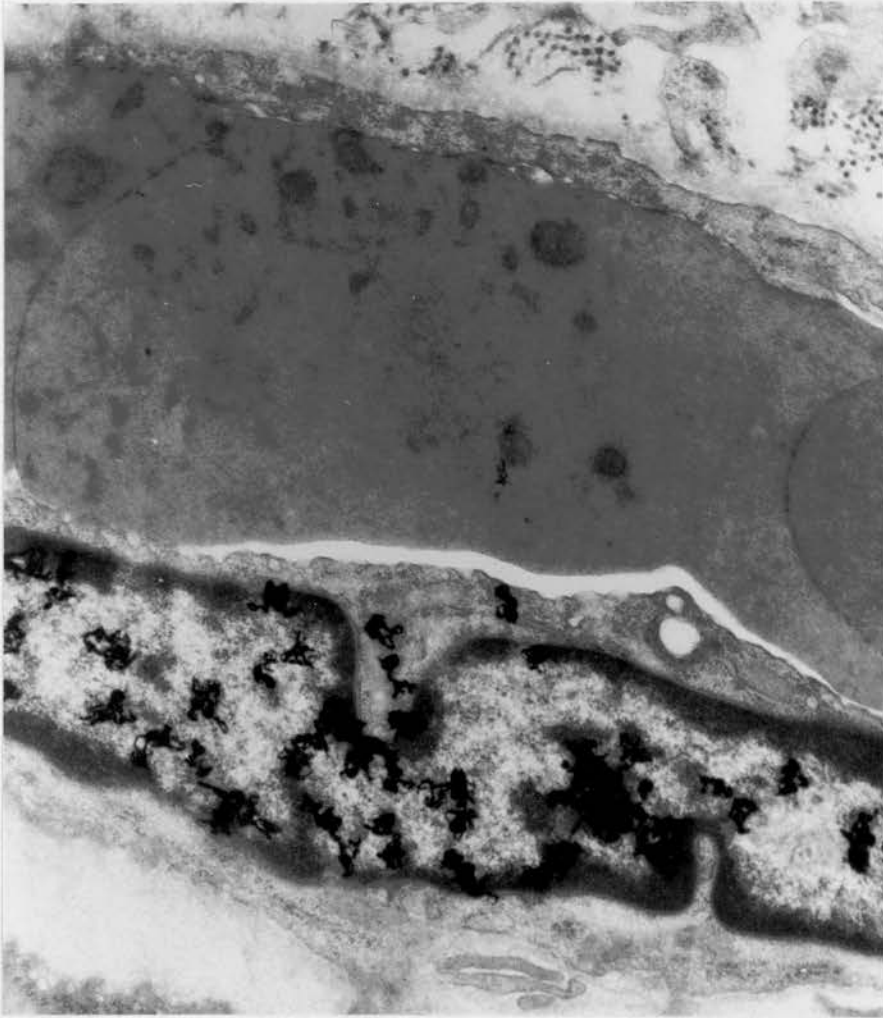


FIGURE 55.

Thymidine uptake autoradiograph of a vascular endothelial cell from the same biopsy as Figure 54, but a thin slice of this tissue was incubated at 44°C. for four hours prior to incubation with tritium labelled Thymidine at 37°C. for one hour. There was no inhibition of Thymidine uptake.

E. SINGLE TREATMENTS UNDER EPIDURAL ANAESTHESIA
FOR THE ARREST OF GROSS HAEMATURIA.

7.28 Early in the initial series it was realised that patients with gross haematuria stopped bleeding following hyperthermia. This was due to the heat induced vascular damage in the vessels of the bladder wall. Following two hours at an outflow temperature of 44°C . there was some frequency which settled after two weeks. This treatment fraction was therefore chosen for the treatment of gross haematuria in that it was the maximum dose which caused vascular damage but did not cause significant bladder contraction.

Method

7.29 The method used was as in the original series. Under epidural anaesthesia the bladder was perfused at an outflow temperature of 44°C . for two hours using a distention height of approximately 20 cms. suprapubically.

7.30 Nineteen treatments were given to twelve patients with gross haematuria, in eleven the haematuria was due to recurrent tumour and in one to post radiotherapy telangiectasia. Five patients were treated twice as the first fraction was unsuccessful, one patient was treated three times.

7.31 In three patients it was not possible to arrest the haematuria. In eight patients the haematuria was arrested for between six weeks and six months, after which time all patients with tumours had recurrent haematuria. The one patient treated for post radiation telangiectasia has remained clear at eighteen months to the time of writing.

SECTION 8.DISCUSSION

- A. METHODOLOGY OF LOCAL HYPERTHERMIA
- B. HYPERTHERMIC PERFUSION OF THE HUMAN BLADDER
- C. ADVANTAGES OF THE NEW METHOD
- D. FRACTIONATED PERFUSION
- E. HYPERTHERMIC IRRIGATION FOR THE ARREST
OF GROSS HAEMATURIA.

A. METHODOLOGY OF LOCAL HYPERTHERMIA

8.1 Heat is an ubiquitous form of energy and many of the problems in localised hyperthermic therapy arise from the difficulty in keeping the heat energy in one place. Short wave diathermy relies on the electrical resistance of tissues through which the current passes. Unfortunately tissue resistance varies with water and fat content and make uniform fields very difficult to achieve. Microwave and ultrasonic therapy is also limited by reflection and refraction in tissues of differing density. The most uniform beam is produced by a very rapidly changing magnetic field. This creates heat by inducing eddy currents and can penetrate tissue evenly because the magnetic properties of tissue do not vary within the human body.

8.2 If such a beam could be positioned at right angles to the plane of a megavoltage X-ray therapy beam a completely variable system could be envisaged. The electrons liberated by photons in the X-ray beam could be accelerated by the changing magnetic field thus altering the effective linear energy transfer in the tumour volume. By varying the heat production, the degree of thermal stress could be changed as could the radiation dose. There is therefore a system which could be tailored to any particular tumour sensitivity with an infinitely variable degree of heat or X-ray damage, and control

of the ability to repair sub-lethal damage.

8.3 Perfusion of a limb isolated from the rest of the body is simple and relies on well known surgical techniques. It has several disadvantages in that it is difficult to give repetitive treatments. A curative dosage has to be given in a single treatment and the normal tissues are therefore pushed to the limit with the resulting 'sick limbs' (Stehlin, 1975).

8.4 Perfusion of hollow organs such as bladder or bowel are simple and do not require major invasive surgery, and the progress and results of such therapy can be observed endoscopically. These features are particularly applicable to the urinary bladder as a considerable amount of work and assessment is done endoscopically in this organ.

B. HYPERTHERMIC PERFUSION OF THE HUMAN BLADDER

8.5 Transitional cell carcinoma of the urinary bladder tends to remain localised and spread mainly by local invasion. In view of the discouraging results following total cystectomy for invasive carcinoma (Whitmore and Marshall, 1962), and the ease with which local hyperthermia can be induced by perfusion it is not surprising that several attempts of this form of treatment have been made.

Local hyperthermic irrigation through a three-way Foley catheter has been used either alone (Hall, Schade and Swinney, 1974) or to enhance the effects of radiotherapy (Cockett, Kazmin, et al, 1967), or chemotherapy (Lungl-mayr, Czech et al, 1973), all with some success but in general disappointing results.

- 8.6 In each of these series the undistended urinary bladder has been perfused. In the contracted state the bladder form folds of mucosa which might shield the tumour from the full effects of hyperthermic perfusion. Distention of the bladder causes full exposure of the tumour and increases the area available for heat exchange
- 8.7 Holden and Gurné (1931) using the Elliot technique in the treatment of gonorrhoea, which involved inserting a bag into the vagina which was then distended and perfused with heated water, reported achieving temperatures of 40°C . in the urethra and 41°C . in the anterior rectal wall.
- 8.8 The present studies were undertaken to assess the validity of hyperthermic perfusion as a treatment in localised cancer of a hollow organ, and to attempt to estimate the depth to which a therapeutic temperature would penetrate.

C. ADVANTAGES OF THE NEW METHOD

8.9 The method of hyperthermic perfusion of the urinary bladder has several advantages over normal perfusion techniques. The apparatus is simple comprising of a drip giving set, a blood warming coil, a paediatric suction trap with a hole bored in the bottom as the overflow tank, a three-way Foley catheter, and a water bath. Distention of the bladder ensures all the mucosa is exposed to the perfusing solution.

8.10 By distention the area exposed for absorption of heat is increased and the thickness of the bladder wall decreased. The pressures per unit area of the bladder wall is related to the height of the overflow tank, and to the bladder capacity. The area exposed increases as a cube power of the radius, as does the pressure per unit area of bladder wall. At full distention this pressure is balanced by the inelastic fibrous coverings of the bladder. Blood vessels are compressed between these two forces and the resultant resistance to blood flow is related to systemic blood pressure and the pressure in the bladder wall. As blood flow is decreased the rate of heat dissipation by blood flow is decreased and conduction through tissue increased. There is therefore greater heat penetration using perfusion

of the distended bladder.

8.11 Muscle tone is absent under high epidural anaesthesia and, as there are no bladder contractions, a steady state of flow is easily achieved. Using a model bladder good and even mixing of the inflowing heated saline was observed.

8.12 The method suffers from any method in which the treatment is applied to one surface in that there is a heat gradient across the tumour. It was hoped that as tumour blood vessels do not vasodilate, the heat conduction would be great enough through the tumour to enable therapeutic temperatures throughout the tumour to be achieved. As the deeper parts of the tumour are intermingled with normal blood vessels which would dilate, we did not expect complete cure, but hoped to be able to destroy the tumour bulk leaving a viable rim which could be treated by local diathermy.

Normal Bladder Sensitivity

8.13 By increasing single fractions of hyperthermic perfusion using an epidural anaesthetic, repeatably effects on tumour and 'normal' mucosa were achieved. The transitional epithelium did not appear markedly heat sensitive, however, by decreasing the blood

flow there were two additional factors which were not expected. The first was local ischaemia and nutritional deprivation sensitising the vascular endothelium to heat damage. The second was physical trauma by compressing vessels and stretching muscle fibres. These factors are assumed to explain the sensitivity of the bladder wall to hyperthermic perfusion during distention.

8.14 A sensitivity graph could be drawn starting with little or no damage up to one hour, then increasing damage with total destruction and fibrous replacement of the bladder wall after four hours treatment at an outflow temperature of 44°C . From the graph of tumour sensitivities (Figure 38), approximately twenty hours at 42°C . or five hours at 44°C . would be required for complete tumour destruction. This was not possible in a single treatment at an outflow temperature of 44°C . without destroying the normal bladder.

8.15 In view of the limiting sensitivity of the vascular endothelium, three patients were treated at an outflow temperature of 43°C . This meant that the heat penetration would be less and that 10 hours would be required for tumour cure. After four hours at 43°C . there was frequency for two weeks but little damage to tumour or normal vasculature. It was therefore apparent that it was not distention alone, but a combination of hyperthermia and distention which caused the limiting

vascular damage. Eventual regeneration of the transitional epithelium occurred in all cases.

D. FRACTIONATED PERFUSION

8.16 A decrease in the inflow temperature had the disadvantages of a poorer heat gradient across the tumour, all of which were T3 extending outwith the bladder wall. After approximately four hours the quantities of Lignocaine required to maintain the high epidural blockade produced some toxicity due to systemic absorption. It was considered that the possibility of treating a patient for ten hours at 43°C . was not really feasible. Fractionation of the treatment into single hourly treatments at daily intervals would enable time for tumour cells to regain sensitivity and treatments might summate unlike the whole body treatments at weekly intervals which did not.

8.17 A planned radical course of therapy of five daily one hour treatments was therefore started. After four such fractions, the patient's symptoms of frequency were slight but the possibility of summation equivalent to four hours at 44°C . in a single treatment was present. The patients were therefore cystoscoped at this stage to assess the degree of bladder damage.

8.18 There was no evidence of summation and the

bladder mucosa appeared as following a single fraction of one hours duration. Microscopical sections taken from the patients at this stage confirmed the clinical impression of no summation during daily fractions. The overall quantity of damage was equivalent to one hours perfusion at an outflow temperature of 44°C .

8.19 Fractionation has been shown to summate to some degree in tissue culture work (Robinson, 1976), but the time between fractions has been of the order of 1 - 2 hours. This is in contradiction to the previous work which showed that following a first exposure to heat, the cells remain resistant to further therapy for a period of up to 17 hours. It may be explained on the basis that, though cell killing is less on a percentage basis, there is an increase in overall cell death which does not occur if sufficient time is left between fractions for complete cellular recovery.

8.20 Our results would indicate that, in vivo, subsequent fractions have to be of a greater degree than previous ones to cause any further tumour necrosis. Multiple fractions within one or two hours of heat other may cause summation but allow some recovery of normal cells. This possibility has still to be explored, but single fractions at daily intervals did not summate.

Fractionated Perfusion without Epidural

8.21 It was believed in this thesis, that hyperthermic perfusion without epidural or general anaesthesia could not be effective. This was because the bladder left with its sensory and neuromuscular reflexes intact, would react to the irritation of local heat by contracting, so decreasing the area for heat absorption, increasing the thickness of the wall and the blood flow per unit area of mucosa. Further, any locally heated area would cause a local contraction of the wall pulling the irritated area out of the heated saline. The mucosa exposed would therefore be constantly changing. This arrangement might explain the unpredictable results which Dr. Hall (1976) was getting with his method, and the fact that he was able to perfuse bladders at an outflow temperature of 45°C . for up to six hours with no residual frequency or side effects.

8.22 It was possible, that by using our method of distention, along with Probanthine to block the neuromotor fibres to the bladder and opiates to decrease pain, that sufficient mucosa should be exposed to allow treatment to superficial lesions. Without epidural anaesthesia the method becomes very simple and easily managed by nursing staff. A further reason for starting a trial of hyperthermic perfusion without epidural anaesthesia, was the possibility that the sensitivity that we

had experienced with epidural anaesthesia was due to bladder damage following the previous radiotherapy, which all our patients had received. The results in this series agree with those of Hall et al (1974) that no frequency or bladder damage, as confirmed by microscopical review of biopsies taken at the end of treatment, is caused by even prolonged therapy without epidural anaesthesia.

8.23 The degree of distention and tone of the bladder, and an active autonomic system, was therefore very significant in altering the effects of a seemingly similar treatment. The possible explanation was that either the thermal gradient was so slight in perfusion without epidural as not to effect the vascular endothelial cells, or that the damage to the endothelium was due to pressure, heat and ischaemia combined.

8.24 Thymidine uptake studies on the transitional cell epithelial cells and vascular endothelial cells were carried out to establish the mode of sensitivity. In vitro studies by incubation of the cells at 44°C. for four hours confirmed that the tumour cells were more sensitive than the normal transitional cell epithelium to hyperthermic stress. There was no inhibition of Thymidine uptake in the cells of the vascular endothelium after incubation at 44°C. for four hours. The vascular endothelium was therefore not greatly sensitive to hyperthermia alone but was sensitised by the

the ischaemia, pressure and trauma induced by the method in association with hyperthermia.

E. HYPERTHERMIC IRRIGATION FOR THE
ARREST OF GROSS HAEMATURIA.

8.25 By limited application of hyperthermic perfusion of the distended bladder under epidural anaesthesia, it was possible to cause thrombosis in the superficial vessels which were bleeding. This principle has been used by surgeons for many years in the form of a hot pack to arrest bleeding. It was found that complete anaesthetic was required to the umbilicus (T10), and a perfusion for two hours with an outflow temperature of $44^{\circ}\text{C}.$, effectively arrested bleeding without causing lasting frequency. Patients who failed to stop bleeding all had markedly reduced bladder capacities, and in those patients the pressure achieved in the bladder wall was presumably insufficient to decrease blood flow to the involved area.

SECTION 9.

CONCLUSIONS

CONCLUSIONS

9.1. Fever therapy has produced some cures in advanced human cancer, but this form of therapy is unpredictable and acts in at least three different ways:-

- a. By direct effect of heat suggested by the finding that the group with the highest degree of fever had the greatest remission rate.
- b. By stimulating the immune response to act effectively against the cancer cells.
- c. By direct toxic effect of the bacterial endo and exotoxins on the cancer cells.

Which of these possible mechanisms is the most important is not known but chemotherapy, immunotherapy and, more recently, heat stress, have all been used individually in the treatment of cancer.

9.2 The effects of hyperthermic stress have been studied by animal and in vitro work which shows that some tumours are markedly more sensitive to hyperthermic killing than their normal counterparts, other tumours are more resistant. Most cellular functions were affected by heat stress to an amount related to the heat sensitivity of the process

studied and the degree of heat stress to which it was subjected.

9.3 Inhibition of oxygen uptake occurred at temperatures above 40°C. in some sensitive tumours and was due to uncoupling of the cytochrome chain with leakage of cytochrome 'C' from the mitochondria. DNA synthesis was inhibited and fragmentation of existing DNA occurred after prolonged heat stress. The nucleolus and RNA synthesis were shown to be particularly sensitive, with failure of formation of active messenger or transfer RNA, and lack of subsequent transport to the ribosomes. Disaggregation of existing ribosomes further decreased protein and enzyme synthesis in the cytoplasm.

9.4 Observations on the cell cycle showed that there was recruitment of quiescent G₀ cells into the cycle, those remaining in the G₀ phase were the most resistant to hyperthermia. Cell killing was greatest in the 'S' phase but cells were also arrested and killed in metaphase. Drugs which blocked DNA synthesis were either protective, or at best, additive to heat therapy, whereas those affecting RNA synthesis potentiated the lethal effects of hyperthermia.

9.5 Membrane permeability increased and was due to a change in the crystalline state of the lipoprotein complexes forming the cellular reticulum. This allowed passive leakage of labelled material across the membrane. Such leakage of lysosomal enzymes, already activated by the decreased pH of

the anaerobically respiring cells, might explain the rapid destruction and absorption of cellular breakdown products reflected in our study. These products would cause fibrin deposition, and, in large amounts could precipitate the disseminated intravascular coagulation state that occurred in some patients. The passive transfer of cytotoxic drugs across these damaged membranes has been shown to occur and may have an important part to play in future chemotherapy of heat sensitive tumours.

9.6 Inhibition of cellular repair systems following hyperthermia might be due to:-

- a. Enzyme usage without replacement.
- b. An increased rate of degradation of existing enzymes.
- c. Lack of energy in the form of ATP to 'drive' the repair system.
- d. Alteration in the quaternary structure of the enzyme, so rendering the 'active site' impotent.

9.7 It was concluded that cellular death following hyperthermia was due to a combination of

damage to nucleic acids, degradation of existing protein with alterations in lipo-protein complexes forming in the cell wall. These changes together with a decreased rate of energy production and inhibition of repair combined cause cellular death. It is probably that different factors are relatively more important in different cell lines.

9.8 Thermal 'tolerance' can be induced in cell cultures by repeated exposure to sublethal doses of hyperthermia. This also occurred following whole body hyperthermia, recurrent tumours proved resistant to further hyperthermia. Fractionation of heat stress was dependant on the survival curve of the tumour as compared with that of the most sensitive normal tissue subjected to heat stress. Above a critical temperature, which was individual for a particular cell line under specific conditions, there was a linear relationship for heat stress between temperature and time. In this range for each 1°C . rise in temperature the time required to produce the same effect was halved. The Centigrade degree minute was adopted in this thesis as the unit of heat stress and was defined as the time equivalent at 1°C . above the critical temperature for a specified cell system under specified conditions required to produce the same degree of thermal stress in that system.

9.9 For whole body hyperthermia a critical

temperature of 40°C . was assumed for all tumours and heat stress worked out in terms of centigrade degree minutes, 41°C . standard. It was concluded that other suggested units such as the 'Thermal Enhancement Ratio' (Robinson and Wizenberg, 1974) were insufficient to characterise the degree of heat stress to which a system is subjected as it does not state temperature, time and critical value of the system. The centigrade degree minute could be modified by potentiation factors and allowed for variations in temperature during treatment.

9.10 Animal studies confirmed the in vitro findings that there was a selective lethal effect of heat on some tumours. These animal studies were carried out using superficially transplanted tumour which could be heated by local methods, such as by immersing a tumour bearing limb in a heated solution. It was found that with the exception of a few very sensitive tumours it was not possible to cause 100% tumour necrosis without loss of the limb. At temperatures above 44°C there was no difference in the rate of cell death between tumour and normal tissue and a temperature of 44°C . therefore represents an absolute maximum for any form of hyperthermic therapy. An animal model for whole body hyperthermia was difficult to achieve as most animals are very sensitive, the normal laboratory animal

the mouse, having a 90% mortality after one hour at a rectal temperature of 41°C . Man was shown to be tolerant to thermal stress when used in the treatment of venereal disease in the 1940's.

- 9.11 Controlled whole body hyperthermia in man by means of immersion in a bath of molten wax proved an effective, cheap and reliable method of rapidly elevating the body core temperature. Introduction of an epidural anaesthetic had the advantages of an early vasodilatation, so increasing the initial rate of heat absorption, a decreased need for systemic opiates and sedatives so that patient's woke up quickly after treatment, and control of heart rate by blockade of the sympathetic outflow to the heart. By leaving the epidural in situ for 24 hours it was possible to use it for post treatment sedation. Ventilation conserved the energy of already weak patients, and by heating the inspiratory gases it was possible to compensate for some of the heat lost by the latent heat of evaporation of water in moisturising the ventilating gasses in the lungs. The method allows for ease of control of body core temperature by varying the area of skin exposed for the evaporation of sweat. Safety is ensured by ease of access to the patient and continuous monitoring of vital functions.

- 9.12 Without epidural anaesthesia there were some-

times alarming rises in the heart rate to over 150 beats per minute. With epidural anaesthesia there was rarely a count greater than 130 beats per minute. It was concluded that the use of epidural anaesthesia was a significant factor in the safety of the method.

9.13 Fluid and electrolyte losses and replacements were great during hyperthermia but by using a standard regime it was possible to keep plasma electrolyte concentrations within normal limits. Besides the electrolyte and sugar infusions it was found necessary to infuse plasma at 41.8°C . to avoid a fall in blood pressure due to expansion of the vascular bed. As the regime is standard it was concluded that renal and sweat functions may be able to balance minor degrees of fluid and electrolyte changes during hyperthermia.

9.14 Liver function was the factor limiting the maximum treatment temperature for whole body hyperthermia. Above 42°C . there were changes in liver function tests with an increase in bilirubin concentration and changes in liver biopsies suggestive of damage (Wills, Findlay and McManus, 1976). At temperatures below 42°C . these changes did not occur and a temperature of 41.8°C . was therefore adopted as the maximum safe therapeutic temperature, leaving a 0.2°C . safety margin.

9.15 Plasma cortisol and ACTH levels showed an

fall due to inhibition of production. Subsequently there was a rise in the plasma ACTH level followed by a steep rise in plasma cortisol. Prolonged treatment resulted in a further rise in both plasma ACTH and cortisol levels suggesting increasing stress to the body.

- 9.16 The white blood count was found to rise during hyperthermic therapy. This has been shown to occur in other forms of pyrexia and appears to be a non specific response of immature polymorphs. No significant depression of the bone marrow was found following hyperthermia, and there was a reticulocytosis between treatments suggestive of an active marrow. It was concluded that the treatment fractions given were not large enough to affect this system.
- 9.17 Treatment fractions were limited by the induction of disseminated intravascular coagulation due to the breakdown and rapid absorption of sensitive cells. This was reflected by platelet consumption, and in more serious cases, by a rise in fibrin-fibrinogen degradation products. Division of treatment into increasing fractions was necessary to prevent the occurrence of intravascular coagulation, especially in patients with sensitive tumours. A first fraction of less than 300 C.^o mins. (250 for very extensive tumours) with an increment of 200 C.^o mins. for each subsequent treatment was given. Intravascular coagulation was only treatable

by infusing clotting factors and platelets as heparin caused bleeding into the hyperdynamic raw tumour beds.

9.18 Viral studies using antibody titres before and ten days post treatment showed no change. Similarly there was no significant changes in immunoglobulin levels following hyperthermia. It was concluded that these tests were not sufficiently sensitive and further studies on the immune status following treatment have been under taken by Mr. A. P. Gee.

9.19 The clinical results of whole body hyperthermia were difficult to assess with regression of disseminated disease being the main criteria. Previous case notes were often inadequately documented, and there were too few cases in any one group to assess the true palliative potential of the treatment. Patients were divided into broad groups with a view to selecting sensitive groups for further therapy. Tumours of gastro-intestinal origin especially colon, and sarcomas appeared to show the greatest response. Lung tumours, and malignant melanomas showed an intermediate response and there was no response in the genito-urinary tumours treated. Testicular and ovarian neoplasms showed no response whatever to hyperthermic therapy. Palliation in the form of tumour regression, relief from tumour induced

pain and general increase in the quality of life was achieved in a number of patients.

9.20 Tumours showing a favourable response to hyperthermia recurred after approximately three months in a heat resistant form and little or no tumour regression was achieved by treating those recurrences. It was concluded that hyperthermia alone offered a good palliative treatment for some sensitive tumours but that this remission was short lived.

9.21 Therapy with increased fractions for 20 hours as a single treatment would be required for tumour cure and this was not feasible. The possibility of decreasing this time by using whole body hyperthermia to potentiate systemic chemotherapy is an exciting possibility. In our limited experience there have been some very good regressions using this combination and the tumours do not become resistant to the combined therapy.

9.22 The complications following treatment were few, and only one death occurred during hyperthermic therapy. Fractionated treatment has removed the chance of disseminated intravascular coagulation occurring and with the present fluid replacement regime the method is safe. Minor complications include sore throat, pressure sores on heels and head, and circumoral herpes simplex in 50% of cases.

9.23 Local hyperthermic perfusions have the disadvantage of a heat gradient across the tumour.

This gradient can be minimised by decreasing the effect of blood flow cooling by inhibiting blood flow. Blood flow can be limited in limb perfusion by use of a tourniquet, or in the method described for hyperthermic perfusion of the bladder, by pressure in the bladder wall. Inhibition of blood flow causes deficiencies of oxygen and nutrients to the cells and increases their sensitivity to hyperthermia.

9.24 The method used for hyperthermic perfusion of the distended urinary bladder under epidural anaesthesia has the advantages of exposing the whole epithelium to the perfusing liquid. It was cheap and easy to set up, and by using a contrast flow rate, it was easy to maintain a steady state with a stable outflow temperature. The results showed that a predictable degree of damage to tumour and normal mucosa followed each increasing fraction of hyperthermia. Because there was a thermal gradient across the tumour and bladder wall it was not possible to express the results in terms of thermal stress (centigrade degree minutes).

9.25 The thermal gradient was less across extensive tumours indicating a limited capacity for increased blood flow through tumours. In extensive tumours the skin or rectal temperatures rose as high as 40°C. In tumours not invading the tissue adjacent to the thermometer, skin and rectal temperatures were nearly normal.

- 9.26 Side effects of treatment were preportional to the duration of perfusion and were due to the damage caused to the bladder wall by ischaemic necrosis of the superficial layers of the muscle coat. The limiting factor was not the epithelium, which in vitro proved remarkably resistant to hyperthermia, but the sensitivity of endothelial cells lining the blood vessels. Damage to the blood vessels was caused by a combination of heat, pressure, and ischaemia and resulted in vascular thrombosis and ischaemic necrosis of the muscle layer supplied by affected vessels. This was minimal after one hours perfusion at an outflow of $44^{\circ}\text{C}.$, but after four hours perfusion the subsequent ischaemia resulted in contracted, fibrosed bladder with a 50 cc. capacity.
- 9.27 Tumour necrosis following hyperthermic perfusion was related to the duration of treatment. All cases treated were T3 tumours extending to the pelvic wall. The conduction was not sufficient to cause complete tumour necrosis, residual tumour presenting later as recurrent tumour. The period of relief from symptoms varied with the degree of treatment. All tumours treated eventually recurred except for two treated at $44^{\circ}\text{C}.$ for four hours. Both of these men have had urinary diversions and no further symptoms.
- 9.28 Fractionated therapy was tried in an effort

to overcome the normal tissue sensitivity to hyperthermic perfusion. Daily fractions did not summate and the tumour and tissue damage was equivalent to the longest single fraction to which they had been exposed. For summation of fractionated therapy the gap between fractions would have to be considerably reduced.

9.29 Fractionated therapy without epidural anaesthesia produced no effect in either tumour or the normal mucosa. This was thought to be due to self protection from noxious stimulæ by the bladder epithelium which contracted out of the perfusate, so constantly exposing a different part of the epithelium. It was concluded that only by paralysing the normal reflex controls of the bladder could a predictable effect be achieved by hyperthermia alone. Perfusion without epidural anaesthesia is unlikely to penetrate more than a few millimetres of tissue.

9.30 To check the sensitivity of the three major tissues of interest, that is the normal mucosa, transitional cell carcinoma, and vascular endothelial cells, to hyperthermia alone Thymidine uptake studies were carried out following incubation in vitro at 44°C. This showed that neither the normal epithelium nor the vascular endothelial cells were killed after incubation at 44°C. for four hours whereas there was considerable necrosis

in the transitional cell carcinoma. This study confirmed that the vascular damage was not due to heat alone and that the tumour was intrinsically sensitive.

9.31 It had been noticed that during treatment under epidural anaesthesia there was arrest of gross haematuria. After two hours perfusion at 44°C . there was no lasting frequency though there was some degree of vascular thrombosis in the deeper layers of the bladder. Two hours at 44°C . was therefore chosen as a treatment for gross haematuria and was successful if the bladder was not contracted and the epidural produced satisfactory anaesthesia.

9.32 The present method of hyperthermic perfusion of the distended urinary bladder under epidural anaesthesia produced reproduceable damage to both tumour and normal mucosa. Single fractions capable of causing tumour necrosis to any useful depth also caused ischaemic fibrosis of the bladder wall. Fractions of treatment at daily intervals did not summate, and it may be possible to allow recovery of the normal epithelium by fractionating at shorter time intervals, which may cause an increasing effect on the tumour. Local perfusion can only be curative in superficial tumours as the heat gradient does not penetrate more than a few millimetres. In this

it may be of use in potentiating existing local chemotherapy such as epodyl instillations.

- 9.33 The future of local hyperthermia is its use to potentiate either local chemotherapy or to localise systemic chemotherapy to the tumour volume but may also be used to potentiate the effects of localised radiotherapy. Other methods of inducing local hyperthermia, in particular a rapidly changing magnetic field, have great promise for the future.

SECTION 10

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

The work in this thesis was carried out in the Departments of Clinical Surgery and Urology, at the Western General Hospital, Edinburgh, and was, in part, under a grant from the Melville Trust for Cancer Research.

In the section on whole body hyperthermia I am indebted to Dr. R. T. Pettigrew, Consultant Anaesthetist, Western General Hospital, for asking me to investigate the physiological and clinical effects of his method, and for his continued help and encouragement over the past five years. I am likewise indebted to Mr. A. N. Smith, Reader in Clinical Surgery, University of Edinburgh, for his advice and guidance throughout this work.

I am grateful to Dr. N. C. Allan and Mr. P. Webber of the Department of Haematology, also to Dr. D. B. Horn and Miss V. Jenkins of the Department of Clinical Chemistry, Western General Hospital, for their scientific advice and for carrying out the haematological and biochemical tests recorded in this thesis. My thanks to Dr. D. Edmund, Virology Department, City Hospital and University of Edinburgh for carrying out the viral tests.

I wish to acknowledge the help of Mrs. J. Galt, Medical Physicist, with the fluid balance studies, especially collection and assessment of

the sweat data.

In the second section on hyperthermic perfusion of the urinary bladder I am indebted to Mr. W. Selby Tulloch, Senior Lecturer, Department of Urology, University of Edinburgh for his invaluable guidance in all matters relating to this work. In both sections I relied on the enthusiasm of Dr. N. McLean, Consultant Pathologist, Western General Hospital, who reviewed all the histological sections.

I am grateful to Mr. J. E. Newsam and Mr. G. F. Carswell for their encouragement and advice, and to Dr. J. Jacob, Lecturer, Department of Genetics, University of Edinburgh, who carried out the electron microscopy and Thymidine uptake studies.

I am indebted to the many Consultants in Edinburgh who have allowed me time to carry out this work while attached to their Departments. Finally, I would like to thank all the nurses and doctors in the Western General Hospital for giving their continued encouragement and support, and Mr. J. Pizer for his care with the illustrations.

I would also like to pay tribute to our patients who have knowingly undergone an experimental treatment with great fortitude, and have willingly co-operated with all the investigations.

APPENDIX

APPENDIX

Patients Treated by Whole Body Hyperthermia

This table is a summary of the patients treated by whole body hyperthermia and lists their disease, previous treatment, the quantity of heat stress that they were given and the clinical results of therapy. It is added to the text to illustrate the difficulty in assessing the results in a totally new form of treatment.

In this series patients were treated in differing stages of terminal cancer with varying heat fractions judged on clinical state alone. Only late in the series was fractionation employed.

Patients with sensitive tumours treated for prolonged periods tended to develop complications. Only by establishing the maximum safe therapeutic temperature together with the use of the centigrade degree minute (41°C . standard). to fractionate therapy, was a safe reproduceable standard treatment achieved.

NAME	AGE	DIAGNOSIS	PREVIOUS TREATMENT			LARGEST SINGLE FRACTION GIVEN C° MINS 41° Std	FRACTIONATED	RESPONSE					ASSESSMENT	SURVIVAL MONTHS
			SURG	XRT	CHEMO			PAIN RELIEF	COMPLICATIONS	DECREASE IN SIZE	PATH	XRAY		
W.K.	69	Pleomorphic Fibrosarcoma	Yes	Yes	No	300	No	Yes		Yes	No	Lung met disappeared	R	21
L.M.	35	Lymphosarcoma	Yes	Yes	Yes	330	Yes	Yes		Yes	No	Healing of Path fracture	R	20
L.W.	24	Lymphosarcoma	Yes	Yes	Yes	100	No	Yes		Yes	-	-	R	1 week
K.C.	54	Liposarcoma	Yes	No	No	420	No	-	D.I.C.	-	Extensive Tumour Necrosis	-	R	1 day
F.D.	3	Rhabdomyosarcoma	Yes	Yes	Yes	350	No	-		-	-	-	NR	2 days
J.M.	41	Osteogenic sarcoma	Yes	No	No	450	Yes	Yes		No	No	No	NR	21
T.K.	37	Osteogenic sarcoma	Yes	Yes	No	830	Yes	Yes		No	No	No	NR	24
W.K.	54	Fibrosarcoma	Yes	No	No	150	No	Yes		No	No	No	NR	1
A.B.	56	Stomach	Inoperable	No	No	120	No	Yes		Yes	No	No	R	3
A.H.	61	Stomach	Yes	No	No	260	No	Yes		Yes	No	No	R	4
A.W.	52	Stomach	Yes	No	No	520	No	-	D.I.C.	-	Extensive Tumour Necrosis	-	R	1
T.M.	69	Stomach	Inoperable	No	No	240	No	-		Yes	No	No	R	1.5
J.S.	65	Colon	Inoperable	No	No	250	No	Yes		Yes	No	No	R	5
E.S.	51	Colon	Yes	No	No	180	No	Yes		Yes	No	No	R	5 days
R.P.	67	Colon	Yes	No	No	100	No	Yes		Yes	No	No	R	3
A.P.	51	Rectum	Yes	No	No	280	No	Yes		Yes	No	No	R	3 weeks
W.C.	61	Colon	Yes	No	Yes	135	No	No		No	No	No	NR	2
E.M.	62	Colon	Yes	No	No	425	No	Yes	Jaundice	No	No	No	NR	3
A.S.	55	Rectum	Yes	No	No	570	No	No		No	No	No	NR	1
J.L.	40	Melanoma	Yes	No	No	103	No	Yes		Yes	No	No	R	2
J.M.	52	Melanoma	Yes	No	No	480	Yes	Yes		Yes	No	No	R	9
A.M.	27	Melanoma	Yes	No	No	240	No	Yes		Yes	No	No	R	0.75
N.S.	60	Melanoma	Yes	No	No	260	No	Yes		Yes	No	No	R	3
M.M.	52	Melanoma	Yes	No	No	135	No	Yes		No	No	No	NR	1
A.D.	50	Melanoma	Yes	Yes	Yes	210	No	Yes		No	No	No	NR	1
M.G.	65	Melanoma	Yes	No	No	720	No	No	Jaundice ? CVA	No	No	No	NR	2
J.H.	53	Melanoma	Yes	No	No	255	No	No		No	No	No	NR	3
M.W.	49	Adeno Ca Bronchus	Biopsy	Yes	No	180	No	Yes		Yes	No	No	R	4
A.H.	56	Squamous Bronchus	Biopsy	Yes	No	320	'No'	Yes		Yes	No	No	R	6
A.P.	47	Undiffr. Bronchus	Biopsy	Yes	No	120	No	Yes		No	No	No	NR	1
Y.G.	48	Undiffr. Bronchus	Biopsy	No	No	410	No	-	? D.I.C.	-	Extensive Tumour Necrosis	-	?R	1 day
I.C.	36	Teratoma Testis	Yes	Yes	Yes	420	No	-	? D.I.C.	No	No	No	NR	1
P.C.	34	Teratoma Testis	Yes	Yes	Yes	360	Yes	Yes		No	No	No	NR	2
C.H.	39	Anaplastic Ovarian	Yes	No	Yes	250	No	Yes		No	No	No	NR	6
B.P.	52	Adeno Ca Ovary	Yes	No	No	120	No	No		No	No	No	NR	4
D.C.	10	Neuroblastoma	Yes	Yes	Yes	240	No	Yes		Yes	No	No	R	6
N.H.	5	Neuroblastoma	Yes	Yes	Yes	110	No	-		No	No	Haemorrhagic Tumour necrosis	R	6 days
D.F.	6	Neuroblastoma	Yes	Yes	Yes	95	No	-		-	-	Gross necrosis or Tumour	R	2 days
M.E.	48	Breast	Yes	Yes	No	450	'Yes'	Yes		No	No	No	NR	3
C.T.	50	Breast	Yes	Yes	Yes	400	Yes	Yes		No	No	No	NR	3
R.N.	44	Mycosis Fungoides	Biopsy	Yes	Yes	320	Yes	-		Yes	-	-	R	8
V.Y.	47	Nasopharynx	Yes	Yes	Yes	493	Yes	Yes		Yes	No	No	R	6
M.K.	53	Acute Myeloid	Biopsy	Yes	Yes	220	No	No		No	No	No	NR	4 days
H.M.	38	Squamous Bladder	Biopsy	Yes	No	130	No	No		No	No	No	NR	3

R = Responsive
NR = Non Responsive

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PUBLISHED PAPERS

I was instrumental in writing the published papers recorded here, but wish to acknowledge the help of Mrs. J. Galt with some of the biochemical results.

1974 Circulatory and biochemical effects of whole body hyperthermia. British Journal of Surgery. 61, 727-730.

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Clinical Effects of Whole-body Hyperthermia in Advanced Malignancy

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British Medical Journal, 1974, 4, 679-682

Summary

Fifty-one patients in the terminal stages of cancer have been treated with whole-body hyperthermia either alone (38 cases) or in combination with chemotherapy (13 cases). Altogether 227 treatment sessions were held averaging four hours each. The most sensitive tumours were those of the gastrointestinal tract and sarcomas. Breast and genitourinary tumours did not respond, and lung tumours and melanomas were only partially responsive. Major complications were remarkably few.

Introduction

Temperatures in the range of 41 to 42°C have been shown to be lethal to tumour cells but not damaging to normal cells (Cavaliere *et al.*, 1967; Vermel and Kuznetsova, 1970; Overgaard and Overgaard, 1972). Hyperthermia has been applied to human tumours *in vivo* by isolated limb perfusion, either alone or in combination with cytotoxic drugs (Cavaliere *et al.*, 1967; Stehlin, 1969), by whole-body hyperthermia (Warren, 1935; Henderson and Pettigrew, 1971), and by local irrigation (Hall *et al.*, 1974). This paper records the clinical responses of a series of patients to whole-body hyperthermia either alone (38 patients) or in combination with cytotoxic therapy (13 patients). All the patients were in the terminal stages of their disease and unsuited to further treatment by conventional methods.

Method

The method used was that described previously (Pettigrew *et al.*, 1974), in which the narcotized patient is covered with

molten wax at 50°C to prevent evaporation of sweat and insulate the body. The overall effect is to raise the body temperature by 3 to 6° an hour depending on body weight. Previous work has shown that the method is safe for treatment periods up to eight hours provided that the temperature does not exceed 41.8°C and so long as there is adequate replacement of the water and salt lost in the sweat (Pettigrew *et al.*, 1974).

Thirty-eight patients have been treated with hyperthermia alone in 188 treatment sessions and a further 13 with hyperthermia in combination with cytotoxic drugs. In the first group the average length of each treatment above 41°C was four hours, and treatments were given at weekly intervals. In the second group treatment was given in three sessions each separated by three days. The first lasted 90 minutes and the other two four hours. Cytotoxic drugs were given by intravenous bolus injection during the last treatment. Patients with malignant melanoma were given Melphalan 1 mg/kg; the others were given cyclophosphamide 200 mg during the period of temperature rise and fluorouracil 15 mg/kg and vincristine 1 mg at a temperature of 41°C.

The response to treatment was judged favourable if there was weight gain or pain relief plus either regression in tumour size on direct measurement or pathological evidence of necrosis in serial biopsy specimens or radiological evidence of regression. Further evidence of heat-induced tumour necrosis was obtained at necropsy in five of the six patients who died soon after hyperthermia.

Nineteen patients were excluded from the series. Fourteen were treated in the developmental stages of the method when temperatures above 40°C were not routinely used. These patients did not respond, and it is now accepted that temperatures in excess of 41°C are needed (Giovannella *et al.*, 1970). A further five patients with no obviously measurable tumours were treated for symptomatic relief of pain only.

Results

HYPERTHERMIA ALONE

Though the numbers were small tumours of gastrointestinal origin and sarcomas appeared to respond more than genitourinary or breast neoplasms; lung tumours and malignant melanomas showed an intermediate response (table I).

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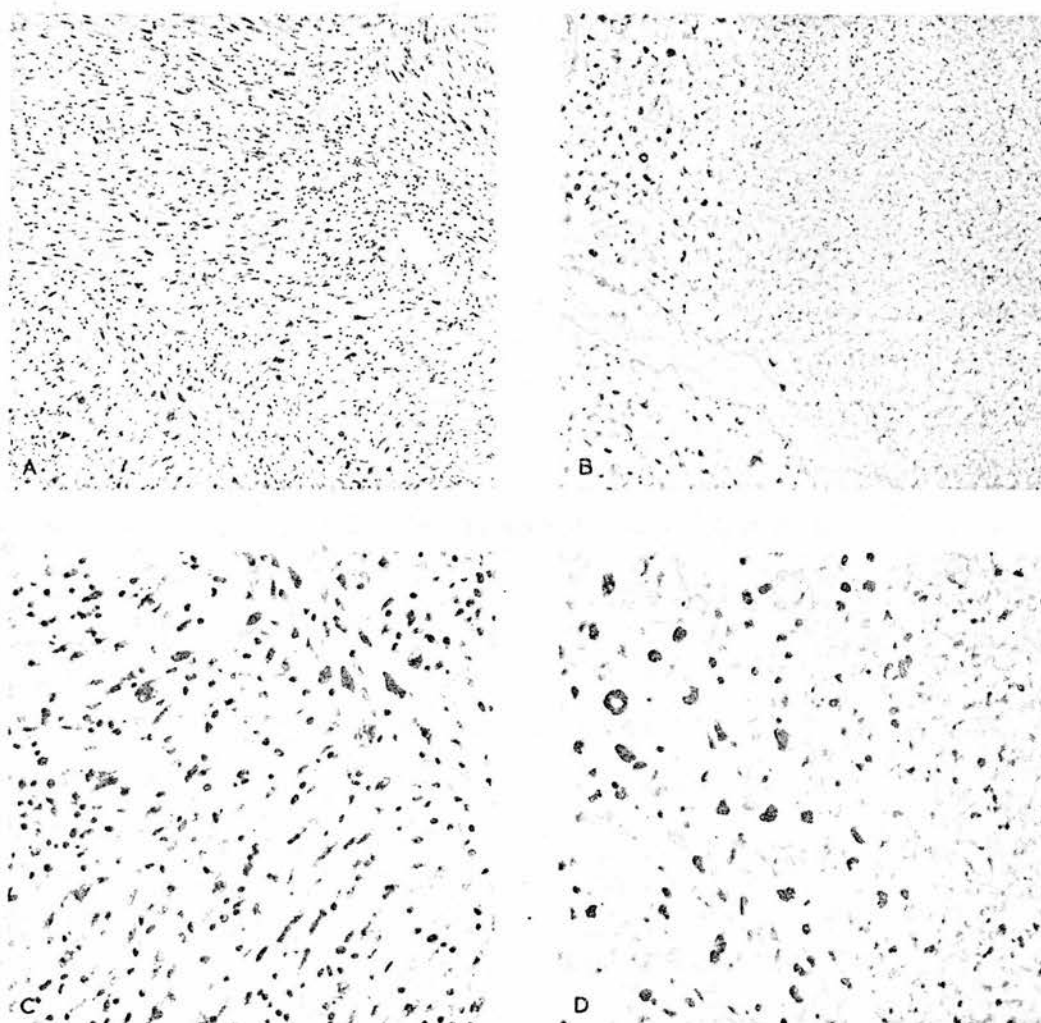


FIG. 1—Photomicrographs of liposarcoma. A and C, appearances of primary tumour after resection. (Haematoxylin and eosin. A $\times 110$, C $\times 270$). B and D, appearances of recurrent tumour four months later after treatment with hyperthermia showing total necrosis to right side and degenerate cells at left margin. (Haematoxylin and eosin. B $\times 110$, D $\times 270$).

Case Reports

Sarcoma.—Eight cases. In one patient a lung deposit disappeared, a second showed healing of pathological fractures, and a third showed complete regression of a fibrosarcoma recurrent in the

operation scar. A fourth had a hindquarter amputation for osteogenic sarcoma; two months after the end of an 18-month course of treatments the tumour recurred in a heat-resistant form. A fifth patient, with an advanced liposarcoma, died 24 hours after treatment. Necropsy showed recent massive necrosis throughout the tumour (fig. 1). Two had subjective improvement with pain relief, and a child with rhabdomyosarcoma showed no response.

Carcinoma of Stomach.—Three cases. Two anorexic patients who had been in great pain gained weight and were able to lead a relatively normal life. The third had extensive mediastinal and lung metastases and died 48 hours after treatment. At necropsy

TABLE 1—Results of Treatment with Hyperthermia Alone

Tumour Type	No. of Patients Treated	Previous Treatment			Objective Responses	Subjective Response	No Response	Survival from Start of Thermotherapy (Weeks)
		Surgery	Radiotherapy	Chemotherapy				
Sarcoma ..	8	6	4	2	3+1*	6	1	84, 52, 20, 8, 4, 2, 1, 0-14
Gastric Carcinoma ..	3	3	0	1	2+1*	2	0	16, 16, 0-28
Carcinoma colon ..	4	4	0	1	2	2	2	20, 8, 4, 0-71
Melanoma ..	7	7	2	2	3	4	3	12, 12, 8, 8, 4, 4, 3
Carcinoma lung ..	3	0	3	0	2	3	0	24, 20, 4
Carcinoma breast ..	2	2	2	1	0	1	1	12, 12
Ovarian carcinoma, teratoma testes	4	4	2	3	0	0	4	24, 16, 8, 4
Neuroblastoma, nephroblastoma	3	3	3	3	1+2*	1	0	24, 0-85, 0-28
Miscellaneous ..	4	2	4	4	1	1	2	32, 24, 12, 0-57

* Necropsy evidence of recent tumour necrosis.

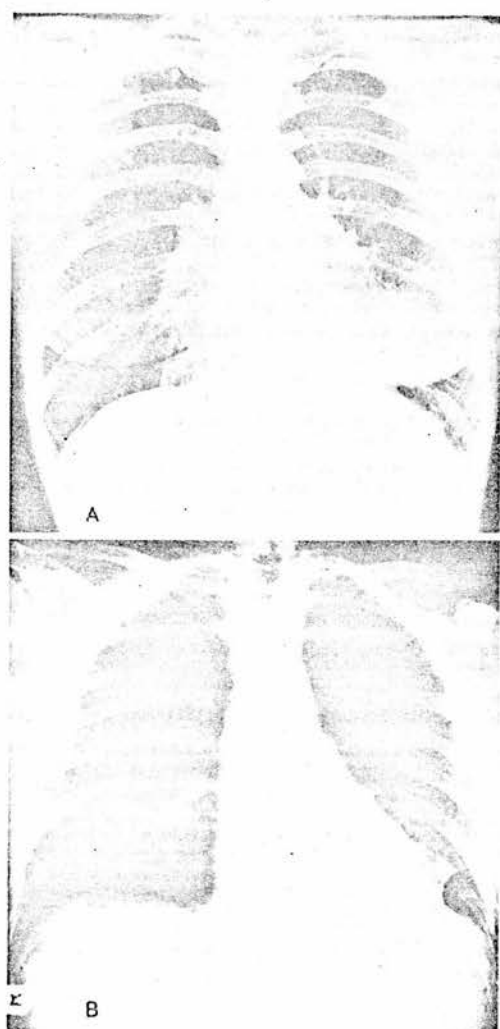


FIG. 2—Chest x-ray pictures from patient with bronchogenic carcinoma. A, appearances before treatment with hyperthermia. B, appearances six weeks later after six treatment sessions.

there was extensive necrosis of the tumour causing compression of the bronchus.

Carcinoma of Colon.—Four cases. One patient had almost complete regression of massive hepatomegaly; a second had regression of skin nodules on the lower abdomen. The other two cases showed no response.

Malignant Melanoma.—Seven cases. In three cases there was good initial regression of secondary deposits and pain relief. One patient had pain relief alone and three showed no response.

Carcinoma of Lung.—Three cases of bronchogenic carcinoma.

In one patient with adenocarcinoma there was regression of the primary lung tumour (confirmed at necropsy) (fig. 2) though the secondary deposits remained active. The second patient, with a squamous carcinoma, showed regression of a secondary deposit in the lumbar spine, and the third had relief from pain but showed no tumour regression.

Breast Cancer.—Two patients with scirrhous carcinomas were treated, one obtaining pain relief alone.

Ovarian and Testicular Tumours.—Two testicular teratomas and two ovarian papillary tumours showed no response to treatment.

Neuroblastoma and Nephroblastoma.—Two children with neuroblastomas were treated. One showed a good initial response, with healing of ulcerated skin over the tumour in his jaw. The second showed initial improvement till he developed respiratory difficulties and died two days after treatment. Necropsy showed multiple haemorrhagic areas of necrosis in the tumour. One child with a nephroblastoma showed initial improvement but also died from a respiratory arrest. At necropsy there was gross necrosis of the tumour.

Miscellaneous Tumours.—One case of mycosis fungoides showed initial healing and there was pain relief alone in a case of adenocarcinoma of the nasopharynx. One case each of chronic myeloid leukaemia and transitional cell carcinoma of the bladder showed no response.

HYPERTHERMIA IN COMBINATION WITH CYTOTOXIC THERAPY

The results in the 13 patients given cytotoxic drugs during hyperthermia are shown in table II.

Case Reports

Gastrointestinal Tumours.—Six cases. One patient had an adenocarcinoma of the colon with large hepatic metastases enlarging the liver to 12 cm below the costal margin. After treatment the liver mass regressed to a lump 5 by 6 cm and the patient was alive and well at six months. The second patient, with a cholangio carcinoma of the liver, showed regression of hepatomegaly with complete clearance of jaundice (initial bilirubin 7.2 mg/100 ml) and resolution of gross ascites. The third patient had an undifferentiated carcinoma and was admitted to hospital as an emergency case with large-bowel obstruction. There was a mass 20 cm in diameter in his left iliac fossa and he had renal failure due to ureteric involvement. After treatment he had complete regression of the mass with a return of normal renal and bowel function. A fourth patient had some regression of hepatomegaly and the fifth showed necrosis on serial biopsy. There was symptomatic improvement in one patient with adenocarcinoma of the gall bladder.

Malignant Melanoma.—Three metastatic cases. Two patients showed regression of involved axillary nodes and the third showed regression of hepatomegaly though her secondary nodules did not change in size.

Breast Cancer.—Three cases. These patients had cancer en cuirasse and were in severe pain. In the first two there was relief of pain with discontinuance of opiates and regrowth of skin over the tumours. One of these patients died within 12 hours of a further treatment given for recurrence three months later. There appeared to be no pathological evidence of tumour necrosis and death was attributed to disseminated intravascular coagulation. The third patient died 48 hours after treatment with disseminated intravascular coagulation. In this case necropsy showed evidence of recent cell death in the tumour metastases, which involved liver, adrenal, both kidneys, skull, uterus, pancreas, vertebrae, and dura.

Miscellaneous.—One case of osteoblastoma was treated. Though the patient had pain relief there was no regression of the tumour.

TABLE II—Results of Treatment with Hyperthermia in Combination with Chemotherapy

Tumour Type	No. of Patients Treated	Previous Treatment			Objective Response	Subjective Response	No Response	Survival from Start of Thermotherapy (Weeks)
		Surgery	Radiotherapy	Chemotherapy				
Gastrointestinal tumours	6	4	1	1	5	5	0	32, 20, 20, 16, 12, 8
Carcinoma breast ..	3	3	3	2	2 + 1*	2	0	20, 12, 0.28
Melanoma ..	3	3	0	0	3	1	0	52, 44, 24
Osteoblastoma ..	1	1	0	0	0	1	0	12

* Necropsy evidence of recent tumour necrosis.

Complications

Complications may arise from the method, from the physiological response to high temperatures, or from the toxic effects of tumour breakdown. When one considers that these patients were maintained in an unconscious state at temperatures of over 41°C for a total of about 1,000 hours complications of a major character were remarkably few. The second patient treated, in 1966, before the present controlled method was evolved, developed ventricular fibrillation. This was due to her temperature reaching 43°C as a result of thermometer failure and was the only fatality directly attributable to induced hyperthermia.

Half of the patients developed a circumoral herpes simplex during the first session but not on subsequent treatments. Sore throats, pressure sores due to prolonged immobilization during treatments, and superficial burns in oedematous, hypoproteinaemic patients occurred.

Four adult patients died within 48 hours of hyperthermia; their deaths were associated with evidence of disseminated intravascular coagulation. In three necropsy showed recent tumour necrosis. Two children with an advanced form of neoplastic disease also died shortly after treatment. They were given opiates for relief of distress and died of respiratory complications. Another patient died of fibrosing alveolitis, possibly due to repeated exposure to the hot, moist, ventilating gases then in use (Henderson and Pettigrew, 1971) or as a result of treatment with bleomycin six months previously.

RECOVERY AFTER TREATMENT

Narcosis is maintained during treatment with short-acting barbiturates and the patient is awake before leaving the theatre. Patients with sensitive tumours show evidence of a systemic reaction after the first treatment, especially if it is prolonged. They develop a persistent tachycardia with a low blood pressure and may remain pyrexial for up to 48 hours. Recovery takes place more rapidly after a subsequent treatment if given within a week. After the first treatment patients may be managed on a day-stay basis, coming into hospital on the morning of treatment and being discharged the next day. Patients with unresponsive tumours show no toxic effects and are fully recovered within eight hours. If treatment is extended beyond four or five hours post-treatment jaundice may develop.

Discussion

There have been no deaths during hyperthermia in over 200 treatment sessions. Of the four adult deaths occurring within 48 hours of treatment all but one were associated with extensive tumour necrosis. In the series of patients treated there were no cures and few complete clinical remissions; however, the advanced nature of the disease in all the patients is emphasized. In general patients responding to treatment experienced a remarkable sense of well-being during the period of remission, with relief of tumour-evoked pain. The quality of life possible after even incomplete treatment of a responsive tumour has been our justification for treating such advanced cases. In doing this it has been established that the selective thermal killing of tumour cells can be extended to human tumours in vivo. Most patients treated with hyperthermia alone who showed initial tumour regression had recurrence of the tumour at about three months in a heat-resistant form.

Animal experiments indicate that at temperatures above 42°C normal cells start to undergo irreversible damage (Burger, 1970; Burger *et al.*, 1970). In this series it was found that at 42°C there was a rise in serum enzymes along with a post-

treatment jaundice (Pettigrew *et al.*, 1974). This did not occur after treatment at 41.8°C, which was therefore taken as the maximum permissible therapeutic temperature. There are indications that a treatment period in excess of 20 hours would be needed at 41.8°C to produce total tumour necrosis (Johnson, 1940). This treatment period may become possible with a greater understanding of the physiological processes which occur at 41.8°C. Several workers have claimed that a synergism exists between hyperthermia and certain cytotoxic drugs (Stehlin, 1969; Giovanella *et al.*, 1970). By combining the two treatments it may be possible to produce total tumour necrosis in a shorter time. The rationale behind this, however, has recently been questioned (Palzer and Heidelberger, 1973 a). Though the numbers in this series were small it appeared that there was an enhanced effect when hyperthermia and chemotherapy were combined.

With any treatment regimen aiming at total tumour necrosis in one session the possibility of toxic products causing deleterious effects such as diffuse intravascular coagulation cannot be ignored (Leavy *et al.*, 1970; Peck and Reiquam, 1973). Yet with therapy given in multiple sessions other problems may be encountered. Work with transplanted animal tumours indicates that heating to a degree insufficient to produce a cure has a stimulatory effect on the spread and growth of metastases (Brett and Schloerb, 1962; Dickson and Ellis, 1974) and may allow repair of sublethal damage to the tumour cells to take place (Palzer and Heidelberger, 1973 b). Heat-resistant strains of cultured human tumours have been produced by exposure to sublethal hyperthermic damage (Selawry *et al.*, 1957). In this series some of the patients with responsive tumours who were treated with multiple sessions of heating without chemotherapy seemed to develop less sensitive tumours. Patients who responded to chemotherapy plus hyperthermia continued to respond to further combined treatments given when there was tumour recurrence.

This work was supported by a grant from the Melville Trust for Cancer Research, Edinburgh.

We should like to thank the many consultants who referred cases, in particular Mr. M. A. Henderson, of the Dumfries and Galloway Royal Infirmary, who helped greatly in the early stages of the work, and also Dr. Neil McLean, consultant pathologist, Western General Hospital, for his advice and help in reporting the pathological findings in these patients.

Requests for reprints should be sent to Mr. A. N. Smith, Department of Clinical Surgery, Western General Hospital, Edinburgh EH4 2XU.

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Circulatory and biochemical effects of whole body hyperthermia

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D. B. HORN AND A. N. SMITH*

SUMMARY

The physiological effects of whole body hyperthermia to 42°C have been studied in 12 subjects. There was a sustained increase in the heart rate and an initial increase in the arterial and central venous pressures. Sweat and electrolyte losses were high. The serum bilirubin remained normal if the temperature did not exceed 41.8°C. Above this temperature the serum enzymes lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase rose, as did the serum bilirubin. While the latter effect might be due to liver damage, it could also be related to thermal destruction of red cells. A leucocytosis develops during the hyperthermic state.

THE selective lethal effect of heat on cancer cells is well known (Mondovi et al., 1969a, b; Muckle and Dickson, 1971); a review of the literature has been published by Vermet and Kusnetsova (1970). Localized human tumours have been treated by isolated hyperthermic perfusion (Cavaliere et al., 1967; Stehlin, 1969). To extend this to the treatment of disseminated malignancy a method of inducing whole body hyperthermia has been developed. This paper details the physiological response of 12 patients to extreme hyperthermia during 70 sessions and is an attempt to establish the safety, or otherwise, of the method.

Method

The method is a modification of that described by Henderson and Pettigrew (1971). The patient is premedicated with promethazine hydrochloride, narcotized with intravenous barbiturates and then curarized. He is sealed in a large polythene bag and placed in a specially constructed bath. Paraffin wax (melting point, 43–46°C) heated to 50°C is pumped into the bath around the sealed bag. The wax solidifies around the patient, forming an insulating layer. Ventilation is maintained through an insulated endotracheal tube with an enriched oxygen mixture heated to 80°C. This is the temperature at the top of the tube, where the closed-circuit adapter meets the endotracheal tube fitting. It falls to 45–55°C at the lower end, i.e. near the carina. Moritz et al. (1945) have shown that dry gas at this temperature does not damage the lungs. The body temperature is raised by between 3 and 6 Centigrade degrees/hour, depending on body weight. During the first hour of temperature rise, the temperature in the oesophagus is 1–2 Centigrade degrees higher than in the rectum (Fig. 1). When the oesophageal temperature reaches 41°C,

usually after 1 hour, the liquid wax is removed, leaving the wax which has solidified around the body as an insulating layer. Following this, there is a slowing in the rate of temperature rise to 41.8°C, during which time the rectal temperature reaches that of the oesophagus (about 90 min., Fig. 1). The wax is removed earlier from thin patients in whom the temperature rise is very rapid. By varying the area of skin exposed for evaporation of sweat or the thickness of the insulating layer, the temperature of the patient can be controlled to within 0.1 Centigrade degree.

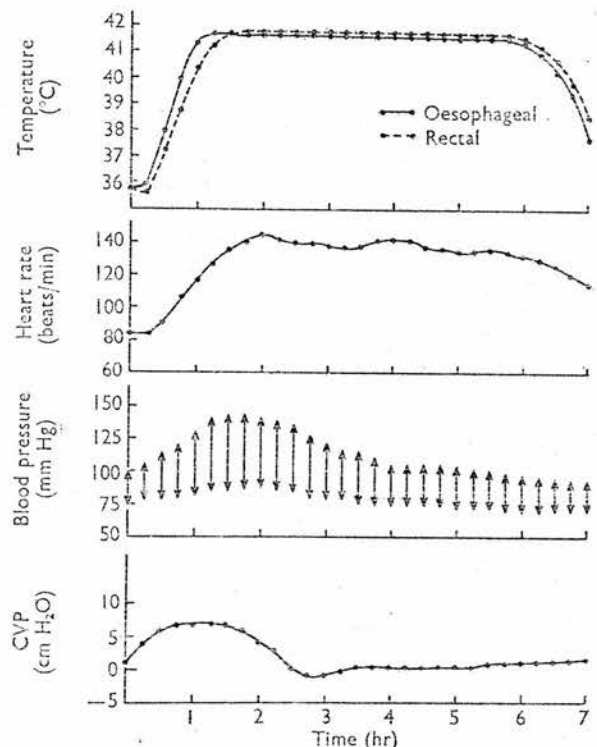


Fig. 1. Haemodynamic response to hyperthermia.

There is continuous monitoring of the rectal and oesophageal temperatures and the electrocardiogram, with periodic monitoring of the blood pressure, central venous pressure, heart rate and serum electrolytes. The patient has a continuous, heated intravenous

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infusion and is given intravenous barbiturates as required. A urinary catheter and nasogastric tube maintain continuous drainage during treatment. When the patient is removed from the bath at the end of the hyperthermic period (at 5–6 hours, *Fig. 1*), cooling is rapid (2–3 Centigrade degrees/hour) and normal body temperature is reached within 2 hours.

Results

Haemodynamic response

A typical haemodynamic response is shown in *Fig. 1*. During heating, the heart rate increases rapidly with temperature. The increase is linear and averages

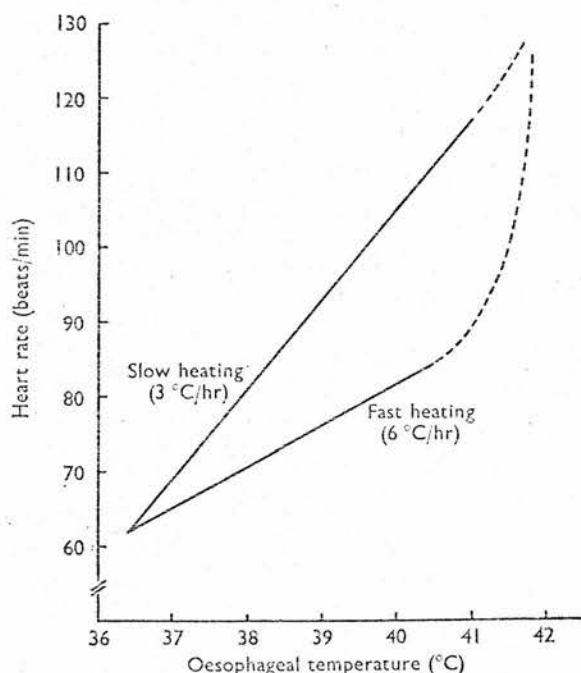


Fig. 2. Variation of temperature-dependent tachycardia with rate of heating.

8.5 beats/min Centigrade degree⁻¹ rise in oesophageal temperature, with a range 5–15 beats/min Centigrade degree⁻¹. In the same patient heated on different occasions the rate of increase of heart rate depends on the rate of heating (*Fig. 2*). Fast heating initially increases the heart rate less per degree rise in temperature. However, at the end of active heating the heart rate continues to rise for some time, reaching a typical maximum for each patient and independent of the rate of heating. The final heart rate at stable temperature corresponds to a rise of 11 beats/min Centigrade degree⁻¹ (range 8–18 beats/min Centigrade degree⁻¹). Stable narcosis is required to maintain this constant heart rate.

Drugs used in the induction of anaesthesia may induce the initial mild degree of hypotension (*Fig. 1*). Thereafter, during heating, the systolic blood pressure rises by 20–50 mm Hg, with little change in the diastolic pressure. At the same time the central venous pressure rises by 5–10 cm H₂O. When the temperature is stabilized, both arterial and venous pressures return to the initial values which are then usually maintained throughout treatment.

Biochemical and haematological changes

As large fluid and salt losses were expected in the sweat, serum electrolyte concentrations were monitored at half-hourly intervals throughout treatment. Results were obtained within 20 minutes of sampling. Initially, fluids and electrolytes were infused according to the level of the central venous pressure and to the serum electrolyte concentration in the most recent sample. From this, the intravenous régime recorded in *Fig. 3* was developed and was used throughout the 70 treatment sessions of this study. With it, there is no significant change in the serum sodium or chloride concentration during hyperthermia (*Fig. 3*), although there is a slight early decrease in the serum sodium concentration, averaging 4 mEq/l which coincides with, and has been attributed to, premedication and anaesthesia (Stevenson, 1960). The serum sodium and

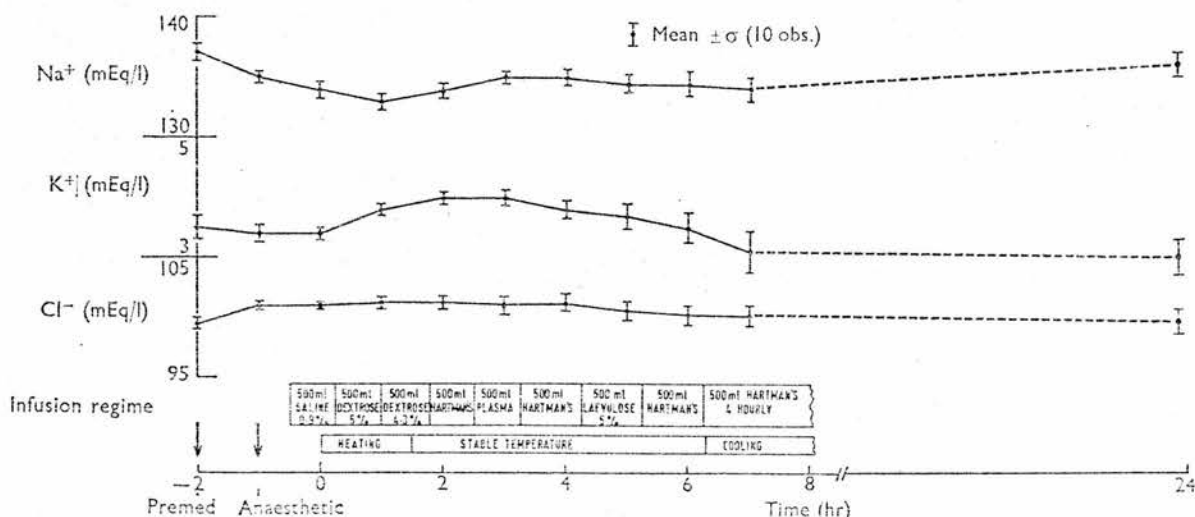


Fig. 3. Plasma electrolyte concentrations during hyperthermia.

chloride concentrations measured 24 hours after treatment show no significant change from the pretreatment values. However, the serum potassium rises by a mean value of 0.5 mEq/l during the first 90 minutes of treatment. This increase is not maintained beyond 3 hours, when a slow fall in potassium concentration starts, and 24 hours after treatment the serum potassium concentration is, on average, 0.5 mEq/l lower than the pretreatment value.

As a check on the accuracy of fluid and salt replacement, losses during treatment were estimated from urinary loss and sweat collection data (Table I). Sweat loss was assessed by enclosing one limb of each patient in a polythene bag and multiplying the volume of sweat collected in the bag during treatment on the assumption that sweat losses from the limbs and trunk are proportional to their relative surface areas. Data given by Allen et al. (1973) indicate that 60 per cent of the sweat is from the arms and legs, including hands and feet, which together represent 59 per cent of the body surface area. Half-hourly sweat samples were also collected from the trunk on weighed pads of filter paper to measure sweat electrolyte concentration and variation in sweat rate. Sweating is profuse during hyperthermia, averaging over 500 ml/hour. The sweat rate increases rapidly with temperature. At stable temperature, fluctuations of up to 10 per cent occur in the sweat rate; no further increase with continued exposure to high temperature has been determined.

The serum activities of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase, all of which have been shown to be stable at the temperatures involved (Burger, 1970), were monitored regularly. In 47 treatments conducted in 10 patients, during which neither the rectal nor the oesophageal temperature rose above 41.8 °C, there was no significant increase in the serum enzyme activities, and the serum bilirubin concentration rose from an average of 0.47 mg/100 ml to an average of 0.62 mg/100 ml, no value lying outside the reference range (Table II). However, during 17 treatments within the same group when the temperature was held between 41.8 and 42.2 °C for periods of 10–40 minutes, the average serum activity of aspartate aminotransferase increased by a factor of 25, that of alanine aminotransferase by a factor of 8 and the serum bilirubin concentration rose to 1.56 mg/100 ml.

During the hyperthermic period the haemoglobin concentration varies about the mean value by up to 0.5 g/100 ml. The haemoglobin concentration falls by an average of 0.8 g/100 ml within 36 hours of each treatment, following which there is a reticulocytosis of 3–4 per cent, leading to partial recovery, but initial values are not attained until the treatment ends. During heating there is a leucocytosis, the average leucocyte count rising from $8.2 \times 10^3/\text{mm}^3$ at 37 °C to $13.7 \times 10^3/\text{mm}^3$ after 4 hours at 41.8 °C. It returns to normal within 24 hours.

Complications

During the developmental stage of the method there were 2 deaths. One was due to ventricular fibrillation

Table I: CONCENTRATION OF ELECTROLYTES IN SWEAT AND URINE LOST DURING HYPERTHERMIA

	ml/hr	mEq/hr			mEq/l			Na : K
		Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻	
Urine*	52	3.3	2.1	3.7	67	51	83	1.3 : 1
Sweat†	514	43	2.0	41	84	3.6	80	23 : 1
Infusion	570	52	1.0	43	—	—	—	—

* Mean of 40 measurements.

† Mean of 60 measurements.

Table II: SERUM ENZYME ACTIVITIES AND BILIRUBIN CONCENTRATION BEFORE AND AFTER HYPERTHERMIA

	Reference range	Before heating	After heating not above 41.8 °C	After heating above 41.8 °C
Lactate dehydrogenase (iu/l)	48–265	181	178	546
Aspartate amino-transferase (iu/l)	4–20	12	13	278
Alanine amino-transferase (iu/l)	2–17	10	12	78
Bilirubin (mg/100 ml)	0.2–0.8	0.47	0.62	1.56

when thermometer failure resulted in a temperature of 43 °C. The other was due to fibrosing alveolitis following repeated exposure to hot moist gases generated by a soda-lime canister (Henderson and Pettigrew, 1971). Dry heated gases have since been used with no harm. These have been the only 2 deaths attributable to the method in a series which now amounts to over 250 treatments, the results of which will shortly be reported. Minor complications experienced were herpes simplex, sore throat with hoarseness, lassitude, nausea and occasional superficial burns under ECG electrodes. There have been no neurological defects or apparent depression of the mental faculties.

Discussion

The hyperthermic state is rapidly and safely induced by this method which effectively reverses the physiological processes allowing heat loss from the body. The solid layer of wax both insulates the body and prevents evaporation of sweat from the skin, while respiratory heat loss is minimized by heating the inspired gases. Knowing the approximate rate of metabolism (1250 cal/min at 37 °C) and the rate of increase of body temperature, it can be deduced that 1000–2000 cal/min are supplied to the body by the change of state of the wax.

The cardiovascular response to increased body temperature found in anaesthetized subjects is different from that found in conscious ones, who respond by a decrease in arterial and central venous pressures due to peripheral dilatation and by a compensatory tachycardia (Damato et al., 1968). A degree of hypotension already exists in the anaesthetized subject before the heating process begins. The rise in arterial and central pressures at the onset of heating may follow a further diminution of afferent

impulses from the baroreceptors in the carotid sinus and aortic arch. This is known to lead to an increase in the activity of the sympathetic vasomotor control of the precapillary resistance vessels which raise the arterial blood pressure and produce increased venous tone and cardio-acceleration. The eventual return of arterial and central venous pressures to their initial values before cessation of hyperthermia is similar to the normal thermoregulatory response to heat stress. The final heart rate at stable temperature represents an increase of only 60 per cent of that found in conscious heat-stressed subjects (Tanner, 1951). This may be attributable to anaesthesia, as a reduction in the depth of narcosis will lead to a further rapid increase of heart rate. The increase in heart rate lags behind the increase in body temperature when the latter is rapidly raised. This may imply that there is a limit to the rate at which the compensatory mechanisms can respond to stimuli.

Fluid losses during hyperthermia are great, averaging over 550 ml/hour, of which 90 per cent is lost as sweat. However, this sweat rate is similar to that reported for unacclimatized subjects exercising in a hot climatic chamber, and although the sweat sodium concentration of 84 mEq/l is higher than the reference range of 20–70 mEq/l, similar increased sweat sodium concentrations have been reported during prolonged heavy sweating in unacclimatized subjects (Furman and Beer, 1963). In spite of the losses it was possible to achieve a satisfactory fluid and salt balance by monitoring the serum electrolyte concentrations.

Raised serum enzyme activities have been found in animals heated above 42 °C (Burger, 1970). This only occurred during this series at temperatures above 41.8 °C, which therefore sets a limit to therapeutic applications. The fall in haemoglobin which occurs following hyperthermia may be due to the thermal destruction of ageing red cells. Reticulocytosis with partial recovery between treatments indicates an active marrow. Similar results were found by Karle (1969) in induced fever in rabbits. The slight post-treatment rise in bilirubin when the temperature has not exceeded 41.8 °C may be due to haemolysis, but alternatively it may be due to liver damage. The development of a leucocytosis during treatment is similar to that seen under stress and may reflect the discharge of additional white cells into the circulation.

Acknowledgements

This work was completed during the tenure of an award from the Melville Trust.

We are grateful to Dr N. C. Allan of the Department of Haematology, Western General Hospital, and to Mrs Anne Tulloch and the nurses of the Recovery Room, Western General Hospital, for their co-operation.

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Coagulation defects following whole body hyperthermia in the treatment of disseminated cancer: a limiting factor in treatment

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(Accepted for publication 6 May 1976)

Experimental studies have shown that some tumour cells may be more sensitive to heat than normal cells. The use of whole body hyperthermia in the treatment of disseminated sensitive human cancer may result in a consumption coagulopathy. Subdivision of treatment into increasing fractions is required to prevent excessive disseminated intravascular coagulation. The consumptive coagulopathy is reflected in a fall in the platelet count. Patients with non-responsive tumours had a fall in platelet count after the first treatment, but not after subsequent treatments. Patients with responsive tumours showed a fall in platelet count after each increasing fraction.

Introduction

Animal and *in vitro* studies have shown that some tumours may be selectively destroyed by heat (Vermel & Kuznetsova, 1970). In man, hyperthermic treatment has either taken the form of localized perfusion for isolated disease (Stehlin, 1969) or of whole body hyperthermia for disseminated disease (Pettigrew, Galt, Ludgate & Smith, 1974b). A method of inducing whole body hyperthermia along with the physiological changes which occurred during treatment has previously been described (Pettigrew, Galt, Ludgate, & Smith 1974a). Single extended treatments carried some mortality, but the method appeared safe for shorter treatments.

Increased intravascular coagulation has been a reported complication of aggressive chemotherapy in the presence of wide spread sensitive tumours (Leavy, Benham, Kahn & Brodsky, 1970). Peck & Reiquam (1973) found that cancer patients have a delicately balanced coagulation mechanism which might easily be tipped towards a low grade chronic disseminated intravascular coagulation by any change in the

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balance. This paper is a summary of the effect of hyperthermia on the coagulation status of 43 patients undergoing whole body hyperthermia for disseminated cancer.

Method

The method used to induce whole body hyperthermia is as described previously (Pettigrew *et al.*, 1974a). The curarized, narcotized patient was placed in a heat sealed polythene bag in a specially constructed bath. Low melting point wax (melting point 35°C) was heated to 50°C and pumped into the bath, immersing the patient. Ventilation was maintained through an insulated endotracheal tube using an oxygen enriched air mixture, heated to 80°C by passage through a heat exchanger. The patients body core temperature was raised through 5°C over approximately 1 h and was maintained at a temperature of 41.8°C during the treatment period. At the end of the treatment session, the patient was removed from the bath and cooling was rapid. Prior to treatment an intravenous cannula was inserted into the median cubital vein and threaded into the superior vena cava. Blood was taken at regular intervals from this cannula before, during and after treatment and the following estimations were carried out.

Haemoglobin, using the Coulter Counter—Model S, Platelet count (Brecher & Cronkite, 1950), using phase contrast microscopy, Quick's one stage prothrombin time using standardized reagents (Poller, 1970), plasma fibrinogen (Ellis & Stransky, 1961) and serum fibrin—fibrinogen degradation products using the Tanned Red Cell Haemagglutination Immunoassay (Merskey, Kleiner & Johnson, 1966). Sternal marrow punctures were performed before, immediately after treatment and at 1 week post-treatment. Three patients received controlled low dose heparin throughout the hyperthermic period. In order to standardize the treatment fractions for different temperature time equivalents, the centigrade degree minute (°C min) was taken as the unit of hyperthermic therapy. This was defined as the temperature in centigrade degrees that the body core temperature was above 40°C multiplied by the time in minutes. Most patients were treated by serially increasing fractions at weekly intervals, though some early cases were not managed in this way. The response to treatment was judged favourable if there was a weight gain, or pain relief, plus either regression in tumour size on direct measurement, or pathological evidence of necrosis in serial biopsy specimens, or radiological evidence of regression (Pettigrew *et al.*, 1974b). The patients form part of a larger series of 64 patients. The results of part of this series have already been published (Pettigrew *et al.*, 1974b); sarcomas (4 out of 8) and tumours of gastrointestinal origin (8 out of 13) were the most sensitive. Melanoma and lung tumours occupied an intermediate group, and there was no response in the ovarian or testicular tumours treated. There was no selection of patients for the present study. They were mainly patients who presented in the latter part of the series.

Results

The most sensitive index of coagulation defect was found to be the platelet count. Other indices, such as prothrombin time, plasma fibrinogen, and the level of fibrin—fibrinogen degradation products, remained stable unless there was a marked thrombocytopenia with platelet counts falling below $50 \times 10^9/L$ following treatment. The

maximal fall in platelets occurred at approximately 24 h after treatment (Figure 1). Sternal marrow histology, before, immediately after treatment and at 7 days post-treatment, in 14 patients, showed no change with no evidence of marrow depression.

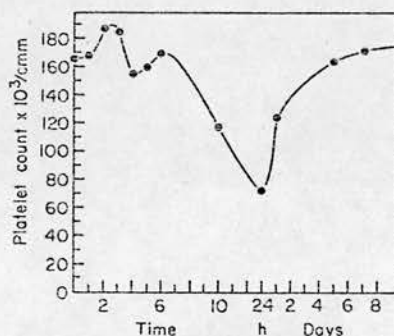


Figure 1. Typical pattern of platelet count changes following a treatment session lasting 4 h (approx. 300°C min).

There was a fall in haemoglobin with a subsequent reticulocytosis following each treatment, as previously recorded (Pettigrew *et al.*, 1974a). There was an initial fall in the percentage of platelets after the first treatment of less than 300°C min, this was less in patients with non-responsive tumour, averaging 75.7% of the pre-treatment value (standard error 13.5%), as compared with those in the responsive group who averaged 45.9% (standard error 9.4%). Both groups showed some decrease in the platelet count following treatment (Figure 2). This was clinically reflected in the responsive group who felt unwell and were usually sick for 48 h following treatment.

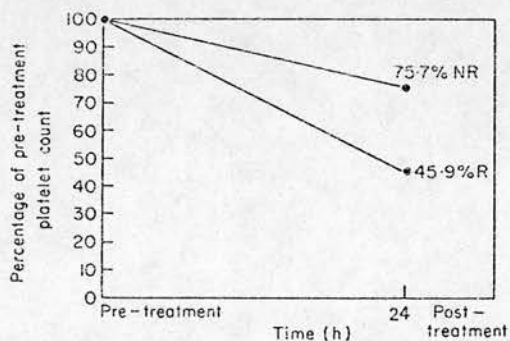


Figure 2. Average results for 15 treatment sessions in responsive (R) and non-responsive (NR) patients, showing a significant difference ($P < 0.01$).

After an initial fraction of greater than 300°C min, there was a greater fall in the platelet count in both the non-responsive and responsive groups. In the non-responsive group the platelet count fell to 74.5% (standard error 17.9%) of the pre-treatment value and in responsive patients, to 17.7% (standard error 11.7%) (Figure 3). There was evidence of disseminated intravascular coagulation (DIC) in the most responsive tumours. Three such patients died with platelet counts below $10 \times 10^9/L$ and fibrin-fibrinogen degradation product levels of 160, 640 and 160 mg/L, respectively, and at post-mortem there was evidence of gross tumour necrosis.

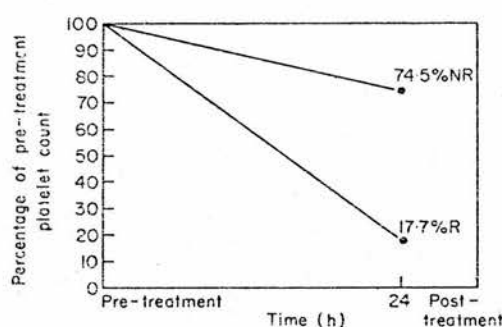


Figure 3. Average results for 11 treatment sessions in responsive (R) and non-responsive (NR) patients, showing a significant difference ($P < 0.01$).

Subsequently, the policy was changed to a first treatment dose of less than 300°C min and no further case of severe DIC occurred.

If a separate second fraction was given above 300°C min , but less than 500°C min , after a first fraction of less than 300°C min , there was a rise in the platelet count in those with non-responsive tumours, averaging 116% of the pre-treatment count (standard error 27.7%); whereas those responding again showed a decrease following treatment, averaging 58.6% (standard error 17.5%) (Figure 4). Two patients with non-sensitive tumours were treated at above 500°C min , following a previous fraction below 300°C min . There was again a fall in platelets to $31 \times 10^9/\text{L}$ and $70 \times 10^9/\text{L}$, respectively; the patient with the greater thrombocytopenia required clotting factors.

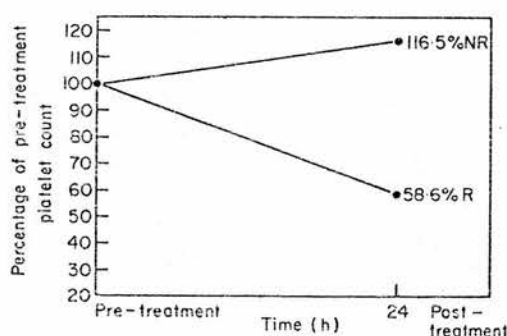


Figure 4. Average results for 11 treatment sessions in responsive (R) and non-responsive (NR) patients, showing a significant difference ($P < 0.05$).

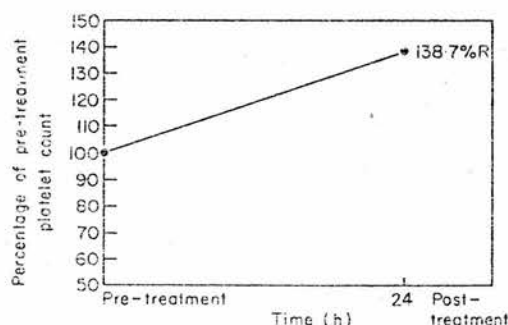


Figure 5. Average results for 4 patients with responsive tumours.

The platelet counts of neither of these two patients returned to their pre-treatment values within 7 days. When patients were treated with the same or a lesser fraction than previously, at an interval of 1 week following the previous treatment, there was an increase in the platelet count in the responsive group, averaging 138.7% (standard errors 57.6%) (Figure 5). These four patients had no nausea or sickness following this fraction. One patient with a non-responsive tumour was also treated in this manner and showed a similar rise in platelet count. In the patients with non-responsive tumours treated at above 500°C min, there was a marked thrombocytopenia and this may represent too large an increment in fraction dosage (200°C min) or a ceiling for maximal treatment.

Discussion

The lethal effects of hyperthermia occur at above 40°C with a decrease in oxygen consumption of the cell (Johnson, Margottini, Mondovi, Moricca & Rossi-Funelli, 1967). Animal studies would indicate that a 1°C rise in temperature results in a twofold increase in the rate of destruction of cells (Westra & Dewey, 1971). The degree minute was taken as the unit of therapeutic hyperthermia and this was defined as the time in minutes multiplied by the temperature in centigrade degrees that the body core is at a temperature above 40°C and represents an equivalent time in minutes spent at 41°C. At a hyperthermia fraction of less than 300°C min, there was a fall in platelets in both responsive and non-responsive patients. There was, however, no subsequent fall in the non-responsive group to a second, larger fraction, 1 week later (Figure 4). This would indicate some initial destruction of normal tissue and may be in part due to the destruction of ageing red cells, as shown in the animal (Karle, 1969). Subsequent greater fractions can be tolerated following a first smaller fraction. The shape of the survival curve of tumour cells during hyperthermia has been shown to be similar to that following X-ray irradiation, having a shoulder indicating repairable damage before an exponential phase of cell death is reached (Westra & Dewey, 1971). The action of hyperthermia in destroying cells is largely by damage to enzyme systems necessary for the repair of otherwise sub-lethal damage (Hahn, 1974). Cells are either killed or return to their previous state following each treatment. Clinical results reflected the platelet count, in that patients having a second fraction, similar to the first do not have a large fall in platelet count and feel well immediately following the second treatment. Each treatment, at weekly intervals, was not additive and a second longer fraction was required to produce the same fall in platelet count. Assuming that the fall reflects effective therapy. This may be due to the remaining population being more resistant.

Cytotoxic chemotherapy and hyperthermia have been shown to potentiate each other (Hahn, Braun & Har-Kedar, 1975). The only death associated with DIC in a patient treated with an initial fraction of less than 300°C min, occurred following chemotherapy given at the same time as hyperthermia. In subsequent cases this combination has been used after two fractions giving the chemotherapy along with the third hyperthermia fraction, of the same dose as the second. Three such patients were treated with closely monitored low dose heparin, during each of the three treatment sessions to try to avoid platelet consumption. One patient with a sensitive tumour bled into her tumours during treatment and this form of preventive therapy

was therefore discontinued. In two patients treated with over 500°C min, in whom DIC was suspected because of rapidly developing thrombocytopenia, treatment by infusion of platelets and clotting factors was successful.

The present results would agree with previous work, that the most useful indicator of acute DIC in patients with cancer may be thrombocytopenia alone. The fact that this may not be associated with reduced clotting factor levels may be explained by an increased production of consumable factors in cancer patients (Peck & Reiquam, 1973).

During hyperthermia the patients are in a hyperdynamic vasodilated state and there is a consequent rapid absorption of any toxic breakdown products resulting from cellular death. This may result in rapid removal of fibrin-fibrinogen degradation products and explain why higher levels were not more frequently found in association with a drop in platelet count. There is some breakdown of normal, probably ageing tissue during hyperthermia but if the load of toxic products is increased by the additional breakdown of sensitive tumour, then disseminated intravascular coagulation may be precipitated. The present results represent a random selection of tumour types which may have differing sensitivities to hyperthermia. All tumours were disseminated and some of the results may relate in part to the bulk of tumour within the body. It is therefore difficult to make a definite clinical evaluation but the trend is towards a consumption coagulopathy in patients with sensitive tumours. There is therefore a need for fractionation in hyperthermic therapy, but the frequency of treatment and the exact heat fractions to be used for each individual tumour are still to be assessed. It would seem that the platelet count may provide a useful measure of tumour response to whole body hyperthermia.

Acknowledgements

This work was supported by a grant from the Melville Trust for Cancer Research, Edinburgh.

We would like to thank Dr N. C. Allan, Haematology Department, Western General Hospital and Miss G. Kerr, Lecturer, Radiotherapy Department, Western General Hospital, for their help and advice.

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Hyperthermic Perfusion of the Distended Urinary Bladder in the Management of Recurrent Transitional Cell Carcinoma

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Temperatures above 40°C have been shown by *in vitro* and animal studies to be selectively lethal to cancer cells (Cavaliere *et al.*, 1967; Dickson and Shaw, 1972). Hyperthermic therapy in man has either taken the form of isolated limb perfusion for localised disease (Stehlin *et al.*, 1975) or whole body hyperthermia for disseminated disease (Pettigrew *et al.*, 1974). Local hyperthermia may have a role in treatment of transitional cell carcinoma of the urinary bladder since the disease tends to remain localised and spreads mainly by local invasion. In view of the discouraging results following total cystectomy for invasive carcinoma (Whitmore and Marshall, 1962) and the ease with which local hyperthermia can be induced by perfusion, it is not surprising that several attempts of this form of treatment have been made. Local hyperthermic irrigation through a 3-way Foley catheter has been used either alone (Hall, Schade and Swinney, 1974) or to enhance the effects of radiotherapy (Cockett *et al.*, 1967), or chemotherapy (Lunglmayr *et al.*, 1973). In each of these studies the undistended urinary bladder has been perfused. In the contracted state the bladder forms folds of mucosa which might shield the tumour from the full effects of hyperthermic perfusion. This is a report of 13 patients with transitional cell carcinoma of the bladder, recurrent following radical radiotherapy, treated by hyperthermic perfusion of the distended urinary bladder. Most presented with cross haematuria not controlled by repeated local cysto-diathermy.

Method

15 treatments were carried out in 13 patients. It was found that even slight bladder distension proved uncomfortable and because of the variation in flow rate due to bladder contraction the temperature was difficult to control. Continuous epidural anaesthesia allowed relaxation of the bladder and a steady flow rate to be achieved at a constant temperature.

Under epidural anaesthesia the nature and extent of the tumour was established by cystoscopy and bimanual examination. Biopsies were taken from the tumour and from the normal bladder mucosa. The bladder capacity was measured and a 24 F 3-way Foley catheter introduced into the bladder and inflated with 5 cc of warm saline. The bladder was then washed out to remove air bubbles and perfused with normal saline which was heated by passing through a heating coil inserted in the water bath. The outflow ascended a gradient to an overflow tank (Fig. 1), the height of which was adjusted so that slightly less than the known bladder capacity caused overflow. This was usually at a height between 15 to 20 cm above the symphysis pubis. The patient was placed on an X-ray table during treatment and the degree of distension was monitored radiographically by injecting a coloured solution or radio-opaque medium into the perfusate and taking check films. Thermo-couples were used to measure the inflow, outflow and rectal temperatures. A further probe was attached to the skin 1 inch above the symphysis pubis (Fig. 1). It is hoped that a further probe, when it is available, will be introduced into the tumour itself to determine

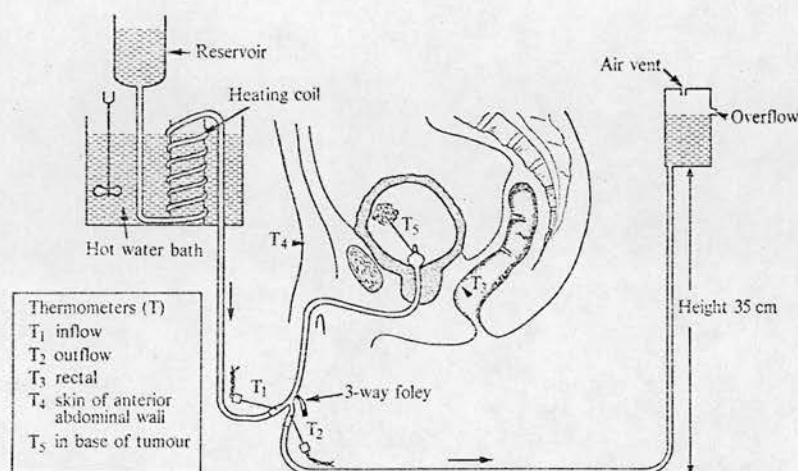


Fig. 1. Method of hyperthermic perfusion of the distended bladder. The overflow height was approximately 35 cm above the table or 15 to 20 cm above the symphysis pubis.

whether the outflow temperature truly reflects the temperature of the tumour. Perfusion was at a constant rate of 3 litres per hour, the inflow temperature being adjusted to give the required outflow temperature. It was initially intended to use an outflow temperature of 45°C (Hall *et al.*, 1974) but this caused lower abdominal discomfort and the temperature was therefore reduced to 44°C, which was well tolerated by all patients. 3 patients were treated at an outflow temperature of 43°C for 4 hours. At the beginning of the perfusion there was a rapid rise in the outflow temperature which remained stable provided that the inflow temperature was kept constant (Fig. 2). The rise in skin and rectal temperatures depended to some extent on the size of the tumour. It was greatest when the tumours were infiltrating and extensive, rising to 40.5°C in such cases, during the first 2 hours of treatment.

At the end of the treatment session a Foley catheter was inserted and left on free drainage for 24 hours before removal. The effect of the treatment was assessed by cystoscopy, bimanual examination and biopsies. These were initially taken 3 days and 2 weeks after treatment and thereafter at 6-weekly intervals. In later cases biopsies were also taken immediately at the end of treatment.

Results

The duration of perfusion varied from 1 to 4 hours. In all cases gross haematuria was arrested but slight haematuria persisted for up to 1 week following treatment. After treatment of 1 hour's duration there was no frequency. At cystoscopy the mucosa appeared healthy and the bladder capacity was not altered but there was residual viable tumour present. These 2 patients had recurrent haematuria at 6 weeks and required further treatment (Table).

After 3 hours at 44°C there was obvious tumour necrosis appearing at cystoscopy as a grey-white slough which took up to 2 months to separate from the mucosa. The normal mucosa appeared red and inflamed and the bladder capacity was reduced to between 150 to 200 ml. There was no evidence of recurrent tumour for 3 months. Thereafter, recurrence was found in 3 of the 6 patients (Table).

After 4 hours at 44°C there was prolonged and continuing frequency and at cystoscopy the bladder capacity was reduced to between 50 to 75 ml. The whole of the bladder mucosa was covered by a grey-white slough. Bimanual examination revealed an indurated palpable bladder.

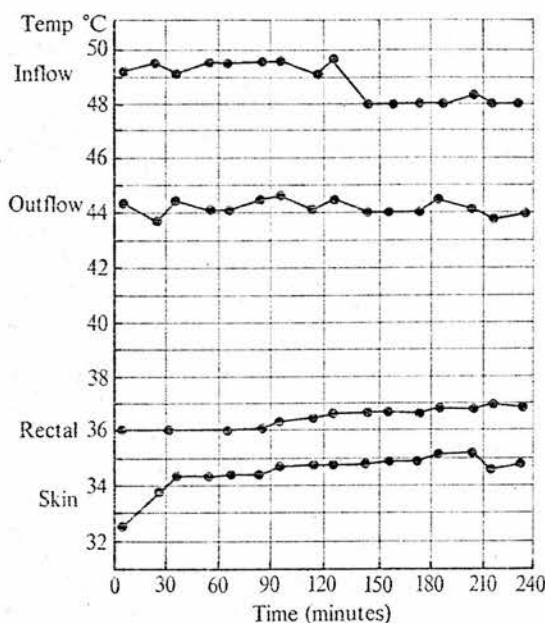


Fig. 2. Temperature graph during treatment.

No recurrent tumour has been found in this group at 6 months but, because of continued frequency, urinary diversions became necessary. They remain well at present with no tumour recurrence (Table).

Histopathology

The tumour biopsies taken before hyperthermia were graded as described by Bergkvist, Ljungquist and Moberger (1965). 6 tumours were Grade II, one of which was invasive; 6 were Grade III, 5 of which were invasive and 1 was Grade IV and invasive.

The effects of hyperthermia on the bladder vary according to the interval after treatment. Changes seen in the transitional cell at the end of the treatment session consisted of nuclear vacuolation, pyknosis, with vacuolation of the cytoplasm, and reduction of epithelial adhesion. These early changes were similar to those reported in the epidermis after thermal injury (Moritz,

Table

Local Bladder Hyperthermia: Comparison of Clinical Results following Different Temperature/Time Combinations

Outflow temp. (°C)	Treatment time (hours)	Duration of frequency (weeks)	effect on tumour
44	1	Nil	Nil
44	2	1	Nil
44	3	12	Clear 3/12
44	4	20+	Clear 5/12+
43	4	6	Some necrosis

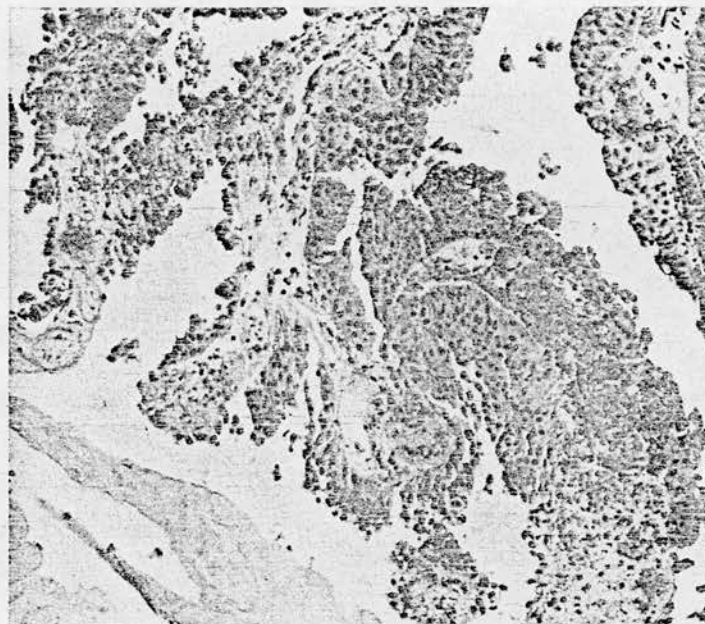


Fig. 3. Necrosis in bladder tumour immediately after treatment for 3 hours at 44°C. Some villi are ghost-like (bottom left). In the central villi, stromal haemorrhage is accompanied by partial necrosis. The remaining villus and its stroma are relatively normal. H.E. $\times 180$.

1947). Slight changes, however, were not easy to assess in a bladder which was inflamed following hyperthermia and had previously been subjected to radiotherapy and repeated cystodiathermy. Severe effects were easily recognised in both tumour and mucosal epithelium and were often accompanied by vascular and connective tissue changes.

Effect of Hyperthermia on Bladder Tumour

No tissue necrosis was seen in tumour biopsies taken after 1 hour's perfusion at 44°C. The effects seen in biopsies taken at the end of 3 to 4 hours' perfusion were often patchy, one part of the tumour showing little change whilst another showed complete necrosis. Necrotic areas were accompanied by local vascular changes such as necrosis and thrombosis in the small vessels of the tumour and haemorrhage into its stroma. These vascular changes may be responsible for some of the tumour destruction since blood vessels are more susceptible to thermal injury than is normal epithelium (Moritz, 1947). Necrosis manifested itself in 2 main forms, either as ghost-like villi or in disintegration of the tumour with disruption of its cells (Fig. 3). 3 days after perfusion for 3 to 4 hours, necrosis was usually so advanced that it was difficult to recognise tumour in the biopsy material.

3 of the tumours recurred 2 months or more after hyperthermia. 1 was a Grade II tumour (Bergkvist *et al.*, 1965) and 2 were Grade III tumours. Both of the Grade III tumours appeared invasive before and after treatment.

Effect of Hyperthermia on Bladder Mucosa

The normal mucosal epithelium initially was considerably less affected than the tumour. Quite marked changes, however, occurred in the blood vessels and connective tissue of the mucosa and these led to additional changes in the epithelium.

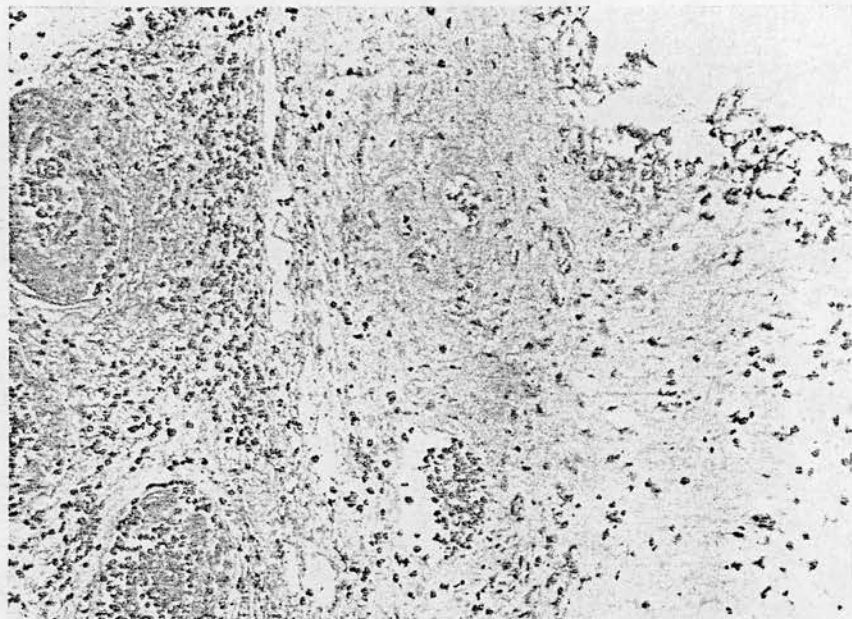


Fig. 4. Bladder mucosa 3 days after 44°C for 4 hours showing vascular necrosis and haemorrhage with devitalisation of stroma and disintegration of transitional cell epithelium. H.E. $\times 250$.

Hyperthermia resulted in congestion and oedema of the lamina propria with inflammatory cell infiltrate sometimes including numerous eosinophils. Thermal effects upon blood vessels of the mucosa led to exudation of plasma and sometimes to interstitial haemorrhage. Some of the vessel walls were frankly necrotic (Fig. 4) and thrombi tended to form (Fig. 5). These vascular changes exacerbated the direct thermal injury of the lamina propria, sometimes resulting in areas of necrosis with exfoliation of epithelium and subsequent formation of hypervascular inflamed granulation tissue. A degree of fibrosis usually followed (Fig. 6). Regeneration of epithelium was obvious in the biopsies taken 2 months after hyperthermia but may have been established sooner.

Discussion

The aim of local hyperthermic treatment of transitional cell carcinoma of the bladder is to cause maximal tumour damage with minimal injury to normal tissue. In this, the method was only partly successful. Distension of the urinary bladder during hyperthermic perfusion ensured that all the papillary tumour was exposed to the perfusing fluid. Continuous epidural anaesthesia prevented intermittent bladder contraction which had previously caused fluctuation in flow rate and difficulty in maintaining a constant temperature. The procedure is simple and was well tolerated by all patients.

Perfusion at 44°C for 3 or 4 hours or at 43°C for 4 hours resulted in tumour necrosis but prolonged perfusion at the higher temperature was followed by frequency of micturition and bladder contraction. This has been found in previous reports (Hall *et al.*, 1974) and may be due to exposure of the whole epithelium or sensitisation by previous radiotherapy. There have been no urinary symptoms following whole body hyperthermia at 41.8°C for up to 11 hours. At temperatures above 42°C there was some damage to normal cells (Pettigrew *et al.*, 1974). In tissue culture experiments some He La cells survive 25 hours' exposure at 42°C; exposure at 43°C produced

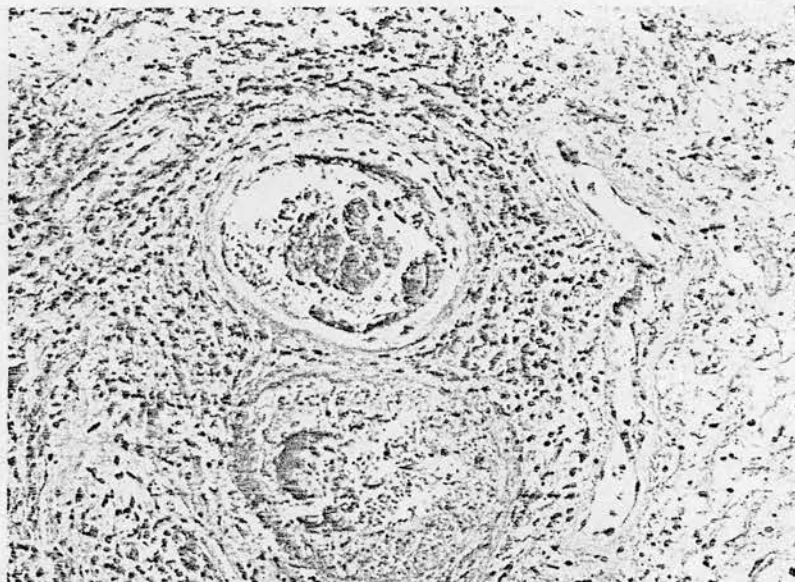


Fig. 5. Bladder mucosa 3 days after 44°C for 1 hour showing non-occlusive thrombi in deep vessels. MSB. $\times 180$.

considerably more cell destruction, few cells surviving 2 hours at this temperature (Palzer and Heidelberger, 1973). The effect of hyperthermia on human and animal tissues is doubled for each 1°C rise in temperature (Moritz, 1947). In the present investigation hyperthermic perfusion for 1 hour at 44°C was not followed either by bladder irritability or by appreciable tumour necrosis. The effect of longer perfusions resulting in tumour necrosis appeared to be brought about not only from a direct action on the tumour cells but also from injury to its blood vessels. This latter mechanism was probably responsible for damage to the bladder mucosa. In the skin, Moritz (1947) has shown that dermal vessels are far more responsive to temperature changes than are normal epithelial cells, and that severe and persistent vascular reactions were often elicited by protracted episodes of hyperthermia of low intensity that fail to harm the epidermis. If this pertains to the bladder it will be a factor limiting the prolonged treatment of tumours deeply infiltrating the bladder wall or surrounding tissue. To achieve a therapeutic effect it is necessary to raise the temperature of an infiltrating tumour to a minimum of 40°C. Prolonged perfusion at 44°C has been shown to produce this temperature in the rectum in the case of extensive tumours infiltrating the bladder base.

Hyperthermic perfusion of the bladder at relatively low temperatures results in necrosis of transitional cell carcinomas and freedom from tumour which may persist for several months. Our results and those of previous workers (Hall *et al.*, 1974) suggest that invasive tumours tend to recur early. Much work needs to be done in terms of sensitivities, fractionation and temperature gradients to assess the role of this method in the treatment of bladder cancer. This preliminary investigation has, however, shown that the method is of value in arresting intractable haemorrhage. Fractionated therapy, at a lower temperature, may cause a selective lethal effect upon the tumour cells without damage to the deeper vasculature of the bladder. It is this possibility which requires further evaluation.

Summary

The clinical and histological changes following hyperthermic perfusion of the distended urinary bladder have been studied in 13 patients with transitional cell carcinoma, persistent after radical

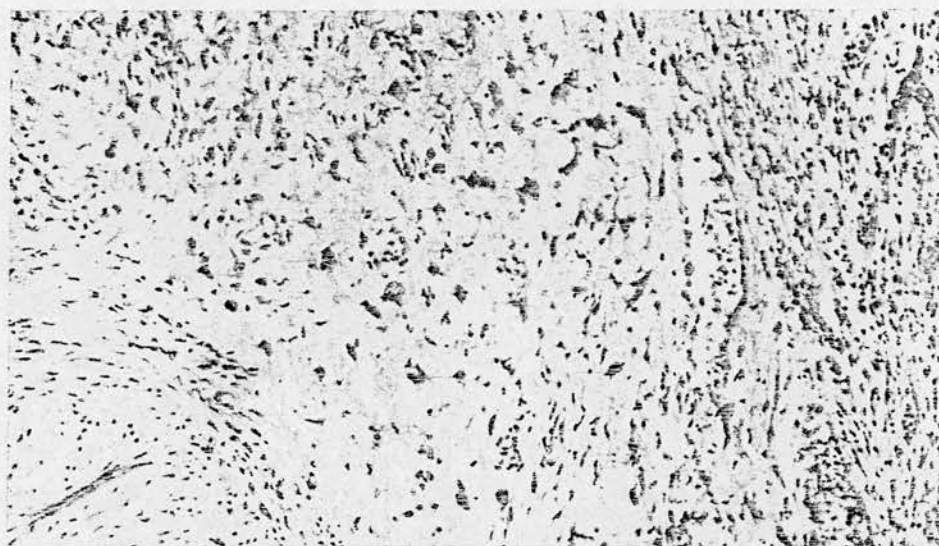


Fig. 6. Fibrosis 2 months after 44°C for 24 hours. The bladder surface is seen bottom right and the muscularis bottom left. H.E. $\times 150$.

radiotherapy. Continuous epidural anaesthesia was necessary to achieve a constant state of bladder relaxation during irrigation of the distended bladder.

This form of hyperthermic perfusion of the bladder was effective in arresting uncontrollable haemorrhage from bladder tumours and may be of value in the treatment of this complication.

Perfusion at an outflow temperature of 44°C for 4 hours caused tumour necrosis. It was, however, associated with damage to the vasculature of the bladder and frequency of micturition which persisted after mucosal recovery. Perfusion at 43°C also caused tumour necrosis and the after effects were less severe.

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